

ASSESSING THE PHOSPHORUS STATUS
OF GROWING BEEF HEIFERS

BY

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Dedicated to

My Mom
with all my love from Pitsy Popper's best friend

My Gramp
If I can be half the person you are,
I will have been a great success

My family (old and new)

My friends

And the fond memory of my friend
Thomas Andrew Shay

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Effects of dietary phosphorus (P) on growth and reproduction and the suitability of various criteria for assessing P status of growing beef heifers were investigated. Relationships between bone mineral content (BMC), cortical area and mechanical properties of bovine third metacarpals (McIII) were defined for this class and age of animal. Additionally, photon absorption, radiographic photometry and ultrasound were tested to determine their ability to predict BMC and bone strength properties of bovine McIII.

The duration of the experiment ranged from 525 to 772 d depending on slaughter date of individual animals. Initially, fifteen weaned Angus heifers were fed ad libitum a low P diet (.10%, dry basis) for 270 d. Heifers were then randomly allotted into two groups (7 animals·group⁻¹) and received either .12 (LP) or .20% P (HP) (dry basis). Heifers were fed ad libitum for the initial 210 d

of this period. Heifers were bred naturally via 120 d exposure to a bull. Common experimental endpoint was day 245 of the treatment phase for nonpregnant and 3 wk postpartum for pregnant heifers.

Total gain and average daily gain were greater ($p < .01$) in HP than LP heifers during the treatment phase (257 vs 205 kg and 1.22 vs .98 $\text{kg} \cdot \text{d}^{-1}$, respectively). Onset of puberty and conception rate were not affected by dietary P level. Five and six heifers in the HP and LP groups, respectively, were found to be pregnant.

Serum, plasma and whole blood P concentrations varied throughout the experiment. Twelfth rib bone biopsies provided the clearest indication of P status of all samples collected in vivo. Rib bone density ($\text{g} \cdot \text{cm}^{-3}$) ($p < .05$) and P concentration ($\text{mg} \cdot \text{cm}^{-3}$) ($p < .01$) were greater in HP heifers.

Breaking load ($p < .05$), breaking strength ($p < .05$) and Young's modulus ($p < .10$) of McIII were all greater in HP heifers. Bone mineral content was greatest ($p < .01$) in each of two 2 cm McIII cross-sections derived from HP heifers.

Correlations between photon absorption, radiographic photometry and ultrasound with BMC of the two 2 cm McIII cross-sections removed at the midpoint and 3 cm proximal to the midpoint were .908 ($p < .0001$), .967 ($p < .0001$) and .565 ($p < .0001$), respectively. Improvements in correlations between BMC and ultrasonic velocity were obtained by using cortical area estimates for distance traveled by generated sound pulses.

CHAPTER ONE INTRODUCTION

A broad spectrum of economic, cultural and technological factors have caused dramatic changes within many segments of the livestock industry over the past 25 yr, especially with respect to practices implemented to increase and maximize production in livestock operations.

Recent advances in such areas as genetic research for increasing economic traits of breeding herds, crossbreeding systems to optimize average genetic merit and utilize hybrid vigor, improved reproductive efficiency (as evidenced by estrus synchronization, pregnancy diagnosis, superovulation and embryo transfer), rumen regulating drugs, implants, feed additives and genetic engineering to produce more useful microflora are just a few of the areas in which scientists have sought methods to increase ruminant productivity.

However, the utility of these advances is doubtful unless the basic nutritional needs of the animal can be met for a given level of production. Therefore, knowledge concerning specific nutrient requirements is a necessity if both economical and optimal beef production are to be realized, as undernutrition is commonly accepted to be the most important limitation to ruminant livestock worldwide.

Phosphorus (P), the second most abundant mineral element in the animal body, has long been recognized for its practical importance in

ruminant nutrition worldwide. Phosphorus is intimately associated with the function of all animal tissues by virtue of its role in the processes of energy exchange. Thus, it may be expected that any limitation to P supply will be reflected in impairment of body function.

Phosphorus deficiency is primarily a condition of grazing ruminants with deficiencies under field conditions having been recognized for many years. Phosphorus has been reported to be the most prevalent mineral deficiency of cattle and sheep in the United States (Mitchell, 1947) and in the world (Underwood, 1981; McDowell, 1985). Because P deficiency is of such great importance, it has been intensively studied for the past 60 yr. Much of the early work on P requirements is still applicable and crucial to current understanding of P nutrition.

Since these early studies, a considerable volume of research has been published on the P requirements of beef cattle. However, there is no clear consensus on the P needs of ruminants. Also, it is obvious that determination of P requirements is only as good as the sensitivity of the response criteria measured. After years of research, reliable and objective methods to determine P status in ruminants are still sought.

The objectives of this study were to compare the influence of two dietary levels (.12 and .20%, dry basis) of P fed to beef heifers in a long-term study on 1) growth; 2) reproductive performance; 3) delineating P status as gauged by various parameters; 4) various

chemical, physical and structural properties of selected bones; and
5) the use of several commercially available noninvasive bone density techniques to predict bone mineral content and bone strength in third metacarpals of these animals.

CHAPTER TWO LITERATURE REVIEW

Introduction

Phosphorus (P), a member of Group V-B of the periodic system along with antimony (Sb), arsenic (As), bismuth (Bi) and nitrogen (N), plays a more varied role in the chemistry of living organisms than any other element (NRC, 1980). Although widely distributed in nature, P is not found in a free elemental state, but rather in the form of phosphates. The earth's crust contains approximately .12% P, whereas the P content of whole cattle is estimated to be .7% (Miller, 1979).

Phosphorus is of great applied importance in ruminant nutrition throughout the world and is probably the nutrient most frequently given as a supplement to grazing ruminants (Cohen, 1980). Deficiencies under practical conditions have been recognized for some time (Henderson and Weakley, 1930; Eckles et al., 1932; Becker et al., 1933). Theiler and Green (1932) cited the observations of LaVaillant in his African travels from 1780 to 1785, which they believed to be the earliest known chronicle of P deficiency signs in cattle.

Mitchell (1947) stated that P deficiency was the most prevalent mineral deficiency of cattle and sheep in the world. Underwood (1966) again pointed out the importance of P in ruminant nutrition

when he stated that ". . . there is no doubt that phosphorus deficiency is the most widespread and economically important of all the mineral disabilities affecting grazing livestock" (p. 34). Blake et al. (1976) concluded that research to better define the dietary P requirement is needed as a failure to provide adequate P to cattle would be deleterious in terms of not only impaired growth and performance, but costly in that providing excess supplemental P to ruminants is substantial and much greater than for any other mineral element. These authors further point out that an overuse of 20%, for example, would mean an unnecessary expenditure of \$80 million annually in the United States.

Metabolic Functions

Phosphorus has more known functions than any other mineral element in the body. Extensive examination of all functions is impractical in the present review; however, it seems appropriate in this study to summarize the major functions.

Phosphorus along with Ca plays an integral role in bone formation and maintenance as well as formation of teeth. Approximately 83% of the P in young cattle is in the skeleton (Duncan, 1958), present as bone mineral (i.e., calcium phosphate $[\text{Ca}_3(\text{PO}_4)_2]$ and hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$) (Harrison, 1984). This percentage is higher in adult than young animals. The large concentration of P within the skeleton provides an excellent storehouse of the mineral which can be mobilized during times of metabolic demand (Guyton, 1981).

Nonskeletal P (approximately 14% of the total body P in cattle) is found in the cell and extracellular fluids as organic phosphoric acid esters, phosphoproteins, phospholipids and the inorganic phosphate ions, $\text{H}_2\text{PO}_4^{-1}$ and HPO_4^{-2} . Although the concentrations of these ions are usually expressed in terms of P content, there is no elemental P in the body as such. Accordingly, some prefer to use the term phosphate in nutrition and metabolism.

The nonskeletal P has many crucial roles in the ruminant animal. It is a key element in energy metabolism as high energy phosphate bonds, such as in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) are essential for providing energy to drive most metabolic reactions.

Nucleic acids, important in genetic and metabolic components, consist of phosphoric acid, a pentose sugar and purine and/or pyrimidine bases. Examples of these include deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

Phospholipids are an important constituent of the cell wall and function in the transfer of materials across the cell wall as well as being important in the transport of lipid materials in blood and lymph. Phospholipids consist of fatty acids, phosphate and a nitrogenous base, such as choline, ethanolamine or serine (Lehninger, 1982).

Phosphorus is an essential component of buffer systems in the blood and other body fluids, including the rumen. Phosphorus is also essential for the proper functioning of rumen microbes, especially

those involved in cellulose digestion (Anderson et al., 1956). Because of its many roles within the ruminant animal body, P is important in virtually every biochemical aspect of the ruminant animal.

Metabolism

Absorption of P from the rumen and omasum is negligible and it has not been clearly shown that P is absorbed from the abomasum (Cohen, 1980). Intestinal absorption of phosphate occurs only in the small intestine, mainly in the proximal (duodenal) end, and both a sodium-requiring active transport process and diffusion are involved (Harrison and Harrison, 1963; Walling, 1977). The precise role of the phosphohydrolases present in the intestinal brush border in the active transport process is not known (Harrison, 1984). Passive diffusion is greatest at high luminal concentrations of P; thus in a P deficiency, absorption will have a greater energy demand on the animal.

Absorption of P is stimulated by the active form of vitamin D, 1,25 dihydroxycholecalciferol ($1,25 (\text{OH})_2\text{D}_3$) (Wasserman, 1981). Wasserman (1981) also reported that the percentage of P absorbed is partially dependent on the level of P intake, but Miller (1983) reported that the percentage absorbed is not closely tied to need as is Ca absorption. Phosphorus absorption percentage seems to be quite variable among individual animals and from day to day for the same animal (ARC, 1980).

The amount of P absorbed is dependent on source, Ca:P ratio, intestinal pH, lactose intake, and dietary levels of Ca, P, vitamin D, iron (Fe), aluminum (Al), manganese (Mn), potassium (K), magnesium (Mg), zinc (Zn) and fat (Irving, 1964; Miller, 1983). Recently, Blum et al. (1974) reported that the effects of high dietary levels of Ca, Mg, Al and Fe are less pronounced in ruminants than simple-stomached animals in regards to decreasing P absorption due to a lower pH in these animals at the site of absorption. Field (1981) reported that absorption of P is largely a function of that part of intake of the mineral which is solubilized in the digestive tract prior to the site of absorption.

Ruminants secrete copious amounts of P in saliva which is in contrast to monogastric animals, with the secretion apparently regulated by parathyroid hormone (PTH) (Tomas, 1974, cited by Cohen, 1980). The daily turnover of P in ruminant saliva is similar to or greater than that of daily P intake (Cohen, 1980). Tomas et al. (1967) reported that total daily P secretion from both parotid glands of sheep exceeded P intake by a factor of 7.2 to 1.3 for P intakes from 0.4 to 4.0 g P·d⁻¹, respectively. It has been estimated that 80% of the phosphate secreted into the digestive tract is salivary in origin (Care et al., 1980), making it the main contributor of P in the gut. The majority of phosphorus in saliva is inorganic phosphate, concentrations of which range from 3 to 20 times that in blood (Preston, 1976). The salts of phosphoric acid in saliva are the most important buffering system in the rumen (Annison and Lewis, 1959). The amount of P in saliva may be involved in the regulation

of endogenous fecal excretion of this element (Miller, 1983).

Phosphorus output in saliva is dependent on P intake, and salivary P concentration is directly related to plasma P concentration (McDowell, 1985).

The rate of salivary flow in ruminants is quite variable. It may be reduced with pelleted diets, concentrate diets and in young animals fed a high proportion of cereal grains. There is generally an inverse relationship between the concentration of phosphate and rate of secretion in saliva; therefore, quantity of P entering the rumen may not greatly decrease when salivary flow is reduced.

The role of the endocrine system in control of P absorption from the digestive tract has been of particular interest in recent years. Fox et al. (1978) have reported that pigs fed diets low in P adapted by a hormonal mechanism such that the efficiency of the mechanism by which P is absorbed (as well as Ca) from the small intestine is increased. Earlier work by Young et al. (1966) indicated that sheep increased the efficiency of P absorption when fed diets low in P. The adaptive change to a diet low in either P or Ca has been shown to be related to increased production of $1,25(\text{OH})_2\text{D}_3$ in the kidney in nonruminants (Hauslar et al., 1976), but has not been clearly demonstrated in ruminants (Care et al., 1980).

As previously mentioned, PTH increases the P concentration in the saliva. The concentration of blood P has no direct regulatory influence on the synthesis and secretion of PTH; however, certain

disease conditions with hyperphosphatemia in both animals and man are associated clinically with hyperparathyroidism. An elevated blood P level may lead indirectly to parathyroid stimulation by virtue of its ability to lower blood Ca according to the mass-law equation when the serum is saturated with respect to the two ions (Krook and Lowe, 1964). Parathyroid hormone is the major regulator of Ca metabolism and maintains plasma Ca concentrations by stimulating bone resorption (Kronfeld et al., 1976). The secretion of PTH is inversely related to Ca levels in the blood that is perfusing the parathyroid gland and is not affected by changes in P levels in the plasma (Sherwood et al., 1968). Cohen (1980) concluded that the major regulatory influence on P turnover was dependent mainly on Ca status of the animal.

The action of PTH on bone resorption is dependent on the presence of $1,25(\text{OH})_2\text{D}_3$ (Omdahl and DeLuca, 1973) and is inhibited by calcitonin (CT), secreted by the thyroid gland (Rasmussen and Tenenhouse, 1967; Guyton, 1981). The active metabolites of vitamin D_3 make bone cells more sensitive to the direct effects of PTH ("permissive effect") and greatly enhance the gastrointestinal absorption of Ca, thus amplifying the effect of PTH upon Ca plasma concentration. Estrogens may also be involved in this mechanism (Guyton, 1981).

Absorption of P from the intestine is enhanced by $1,25(\text{OH})_2\text{D}_3$ as previously noted (Wasserman, 1981), which is a product of

sequential hydroxylation of vitamin D₃ in the liver and kidney. The second hydroxylation which occurs in the kidney (1 α -hydroxylase step) is stimulated by low serum (and intracellular) inorganic phosphate levels and depressed by high serum calcium levels (DeLuca and Schnoes, 1976). Figure 1 is a schematic representation of the hormonal control of phosphorus homeostasis in the ruminant.

In contrast to monogastric animals, ruminants excrete most of the endogenous P through the feces (ARC, 1980); however, a greater amount has been reported in the urine of cattle fed high concentrate diets (Preston, 1976). These findings are related to the fact that ruminants have a higher renal threshold for P than do monogastrics (Mayer et al., 1968). Advantages of this fact for the ruminant lie in their evolutionary adaptation to low quality roughage diets, which generally contain little P. At various times, the amount of P required for salivary production and intake are opposed. If the renal threshold for P was low, then at times when intestinal P reabsorption is occurring, thus causing an increase in plasma P concentration, P would be excreted in the urine. This P would not be "surplus" to the requirement of the animal and would then have to be met by a diet originally low in available P.

Mayer et al. (1966) have shown that PTH decreases the daily excretion of fecal P by cows, although it was not proven if this was due to enhanced P absorption. These cows had a marked increase in urinary excretion of P when given an I.V. dose of PTH. Parathyroidectomy was followed by a decline in urinary P and subsequent rise in fecal P.

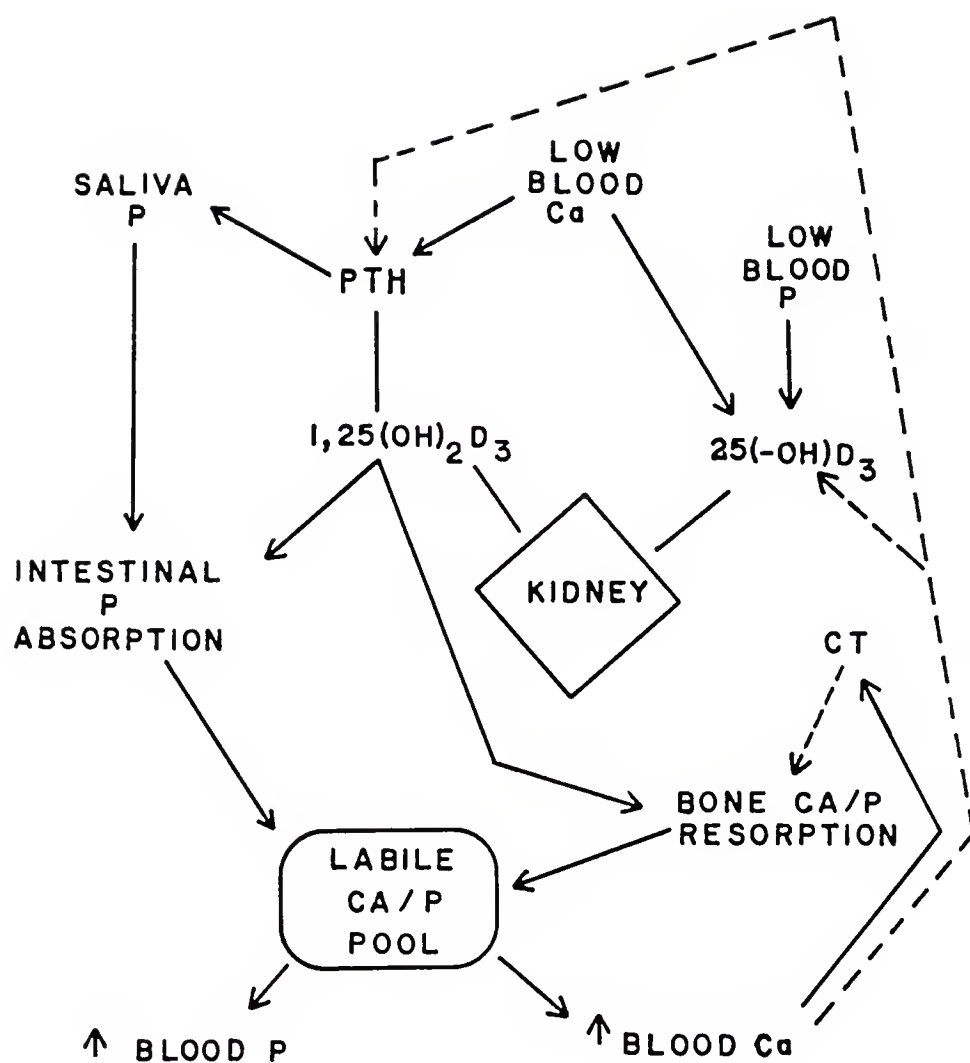


Figure 1. Hormonal regulation of phosphorus homeostasis. Solid line (—) represents positive stimuli, broken lines (---) negative feedback. PTH = parathyroid hormone; CT = calcitonin. Adapted from Cohen, 1980.

Barrow and Lambourne (1962) indicated that organic P excretion remains relatively constant and independent of the amount of P consumed, whereas fecal inorganic P varies directly with P intake in cattle (Cohen, 1974).

Deficiency

Phosphorus deficiency is predominantly a condition of grazing ruminants (McDowell, 1976; Underwood, 1981). Cattle long restricted upon the native forage grown on any fenced pasture area reflect in their physical condition and performance the nutritive value of the forages and the fertility of the soil. A combination of soil and climatic effects on forage P concentrations are the major contributors in defining the P deficient areas throughout the world for grazing livestock (Underwood, 1981). Soils low in plant available P result in forage with below normal P contents and the occurrence of a dry period in each year, when the plants are dry and mature and the seed is shed, exacerbates or prolongs this effect. Whole-plant P contents fall rapidly with increasing maturity; this coupled with low initial P concentrations in the plant and long dry periods will result in low P concentrations in forage for long periods (Underwood, 1981). Additionally, under field conditions, the protein content of the forage generally parallels that of P, such that a protein deficiency, and many times a deficiency of available energy, are all contributing factors in the malnutrition of livestock in P deficient areas (Underwood, 1981; McDowell, 1985).

Pioneer P supplementation studies in South Africa in the early 1900s (Van Niekerk, 1978) revealed the cause of bovine botulism and aphosphorosis. Both conditions were the result of a severe P deficiency, with cattle exhibiting subnormal growth and reproduction and a depraved appetite or "pica" as illustrated by bone chewing. The syndrome of bovine P deficiency, according to Theiler and Green (1932), appears as follows: hypophosphoremia, osteophagia, osteoporosis, osteomalacia (in the adult) or rickets (in the young), cachexia and exitus. Eckles and Gullickson (1927) and Riddell et al. (1934) observed a depraved appetite as exhibited by chewing rocks, soil consumption and chewing stall chains. A general lack of thriftiness and poor condition were also observed. Kleiber et al. (1936) described the signs in beef heifers as similar to those above. Pica was manifested largely as coprohagy and marked osteophagia when tested with sun-bleached bones. Abnormal chewing of objects may also occur with other dietary deficiencies however (e.g., Na and Ca) (McDowell, 1985). Often the pica as represented by bone chewing has been misinterpreted to suggest that P deficient ruminants selected substances containing P. Many materials sought by P deficient animals contain little or no P (Becker et al., 1933; Preston, 1976).

Almost every phase of performance in relation to cattle may be depressed by a P deficiency. In addition to those P deficiency signs previously mentioned, others reported include lethargy, stiffness, decreased voluntary feed intake, lower feed efficiency, reduced lactation, impaired reproduction and bone abnormalities

(Eckles et al., 1932; Becker et al., 1933; Huffman et al., 1933; Preston, 1976; Miller, 1979; Underwood, 1981).

Requirements

Knowledge concerning specific nutrient requirements is a necessity for optimum or economical beef cattle production. A considerable volume of research work has been published on the requirements of P in beef cattle nutrition; however, there is no clear consensus on the needs of ruminants for macro-elements, including that of P (Field, 1981). Braude (1978), addressing this, stated that the best we can expect from requirements is that they provide us with guidelines, which if interpreted intelligently, are useful in practical livestock feeding, but are by no means absolute.

Underwood (1981) reported adequate Ca and P nutrition depends not only upon sufficient total dietary supplies, but also on the chemical forms in which they occur in the diet, vitamin D status of the diet or animal, as well as the Ca:P ratio in the diet. However, other factors that should be taken into account when determining the actual nutrient level in the diet include variability of ingredient nutrients, animal performance potential, energy and protein level of the feed, ambient temperatures, stress (i.e., disease, overcrowding, poor ventilation, inadequate temperature control), nutrient availability, present status or need for the nutrient, compensatory growth, interaction of nutrients, variability in animal response and management variability. It is, therefore, essential that as many

factors as possible be taken into consideration when assessing requirements so that proper interpretations can be made.

The main basis for mineral requirements of ruminant livestock today are the National Research Council (NRC) publications on nutrient requirements of beef cattle (NRC, 1984) and dairy cattle (NRC, 1978), in addition to the British publication on nutrient requirements of ruminants [Agricultural Research Council] (ARC, 1980). The following discussion reviews published results as they pertain directly and indirectly to minimum P requirements in beef cattle nutrition.

Growth

Using a ration calculated to contain .26% P on a dry matter basis (DMB), composed of equal parts of corn and alfalfa hay, Forbes et al. (1929) fed 2 yr old steers four planes of nutrition from one-half through twice maintenance energy intake to establish changing P balance. The steers in this trial were in positive P balance when fed a maintenance energy intake or above, indicative that P requirements relative to energy requirements may be greater for near maintenance compared to fattening in more mature steers.

Kleiber et al. (1936) found that a diet containing .13% P (DMB) was not adequate for beef heifers which ceased to grow after 6 mo, only maintained their weight when P level was reduced to .09% and lost weight when the level was further reduced to .07% when compared to controls fed .4% P. The authors suggested that P deficiency was associated with impaired utilization of energy (i.e., they found that

P deficiency lowers total efficiency mainly by lowering appetite and, secondly, by lowering the availability of metabolizable energy for net energy purposes; digestibility of the feed was not affected). Kleiber et al. (1936) also reported that bone chewing and coprophagy developed in the low P groups. Serum P decreased approximately 67% in heifers fed .07% P vs controls receiving .4% P.

Henderson and Weakley (1930) reported that dairy calves fed .13% P with an adequate level of Ca grew as well as calves fed .22% P. However, lower serum inorganic P levels (3.89 vs 4.69 mg·dl⁻¹) and bone ash (55.7 vs 60.7%, dry-fat-extracted) were observed for the calves fed .13% vs .22% P, respectively. Huffman et al. (1933) reported that .20% P was insufficient for satisfactory growth in dairy heifers from 3 to 18 mo; however, .41% P appeared sufficient. In a similar study with two groups of 6 mo old calves, Archibald and Bennett (1935) indicated that approximately .18% P was as effective as .31% P in promoting growth.

Beeson et al. (1937, cited in Preston, 1976) reported that a ration containing .12% P (DMB) (8.23 g P·d⁻¹) fed to growing calves resulted in poor condition, rough hair coats, wood chewing and soil consumption. They concluded that the "physiological minimum" requirement was .19% P and recommended .21% P (DMB) (2 g P·45.5 Kg⁻¹ body weight·d⁻¹) as the P requirement for growing-finishing calves. Mitchell (1947) stated that a P level of less than .12% (DMB) in forage will not supply adequate P for growth of cattle, consistent with the work of Beeson et al. (1937, cited in Preston, 1976).

Burroughs et al. (1956) reported an increase in feedlot performance of growing-finishing steers when ration P levels were increased from .18 to .25%. In this same study, the authors postulated a higher P requirement for rumen microbes than for the growing host. This was based on the observation that steer calves fed a ration containing .28% P (DMB) gained faster than those fed .20% P, even though blood inorganic P levels were the same. However, it has been reported by South African workers (Clark, 1953) that, even during clinical hypophosphorosis, concentrations of water soluble P in rumen fluid do not fall below $200 \text{ mg} \cdot \text{L}^{-1}$. Preston and Pfander (1964) reported that growing lambs fed rations containing either .12, .15, or .29% P (air-dry basis, 91% DM) had proportional increases in total P concentration in rumen contents (.692, .931 and $1.17 \text{ mg} \cdot \text{g}^{-1}$, respectively) as well as total rumen fluid P (1.37, 2.04 and 3.20 g, respectively). Average daily gain of lambs for the 3 rations was 5, 28 and 168 g, respectively, over the first 49 d of the experiment. Cohen (1978; cited in Cohen, 1980) has reported rumen inorganic P concentrations between 150 and $183 \text{ mg P} \cdot \text{L}^{-1}$ rumen fluid in heifers with low intakes of dietary P (5.0 and $15.3 \text{ g} \cdot \text{d}^{-1}$, respectively).

There are still few reports in the literature on the P requirements of rumen microorganisms, although these levels are in excess of the minimum requirements of $60 \text{ mg P} \cdot \text{L}^{-1}$ for *Bacteroides succinogenes* (Bryant et al., 1959). It has been suggested from in vitro results that a mean level of about $100 \text{ mg} \cdot \text{L}^{-1}$ of available P in the rumen is adequate for growth of rumen bacteria and for

cellulolytic activity (Durand and Kawashima, 1980). The soluble P content of rumen fluid in vivo is usually well above this suggested level unless P intakes are very low (Table 1). It would seem from this discussion that the P requirement of rumen microbes is high; however, satisfaction of the microbial requirement depends more on salivary P, plasma P and bone resorption than on the temporary supply of available P. Witt and Owens (1983) reported no difference between 9.5 or 18.3 g P·d⁻¹ fed to mature cattle on rate or extent of digestion and suggested there was some evidence to indicate that P may be mobilized before digestibility of nutrients is reduced.

Feed intake, weight gains and plasma inorganic P concentration showed linear increases (Long et al., 1956) over the range of .07, .11 and .15% dietary P in yearling steers (177 Kg average initial weight) fed diets containing cottonseed hulls, dried beet pulp, dehydrated alfalfa meal, corn gluten meal and urea. Supplemental P was added as NaH₂PO₄ and Ca:P ratios were standardized over all diets at 4:1. Slight increases in gains were observed when the P level was further increased to .19%; however, this was not significantly different from gain observed in steers fed the .15% P diet. Wise et al. (1958) reported that ruminating calves (91-125 Kg, initially) had a growth requirement of about .22% P, air-dry (.24% P, DMB). These calves were fed a semi-purified basal diet (.09-.10% P, air-dry) composed of corn meal, beet pulp, dried molasses, glucose, corn oil, corn starch, blood meal, urea, minerals and vitamins A and D. Phosphorus in the basal diet was supplemented as dicalcium phosphate. At P levels below .18%, feed consumption and growth were

Table 1. Influence of type of diet and dietary mineral level
($\text{mg}\cdot\text{kg}^{-1}$ DM) on P content ($\text{mg}\cdot\text{L}^{-1}$) of rumen fluid (RF).

Animal	Diet	P	
		Diet	RF
Sheep (4) ^{a,b}	Urea-corn concentrate	3600	1300
	Alfalfa hay	2800	370
Cattle (3) ^b	Milo	800	1243
	Alfalfa hay	400	50
Steer (1) ^c	Grass hay	2900	360
Steer (1) ^b	Mixed grass hay + wheat bran	6800	831
Steers (4) ^b	Mixed hay semi-purified	400	200
		1600	420
		5400	540
Lambs (4) ^b	High beet pulp	1300	690
		1600	930
		3200	1170
Wethers (3) ^b (4) ^b	Semi-purified	0	80
		7000	760

Adapted from Durand and Kawashima (1980).

^a Number of animals.

^b Strained RF.

^c Clarified RF.

depressed. Also calves fed below this level had rough hair coats and were unthrifty as compared to calves fed diets containing .22% P or more. Bone growth appeared satisfactory at the .22% P level, although rib bone ash was numerically higher at levels of .30 and .38% P (48.9, 51.4 and 50.1, respectively). Tillman et al. (1959) fed 18 Hereford steers (146-182 Kg, initially) a basal ration containing .12% P for 47 d. A 70 d experimental period followed with animals assigned by weight and age to three groups: either .14, .17 or .20% P. Supplemental P was provided as dibasic calcium phosphate. Weight gain, feed consumption and feed efficiency increased linearly, suggesting that the .17% P level was not adequate for these criteria. Bone growth and plasma inorganic P, however, differed from linearity in the .17 and .20% P groups indicating that based on these criteria, the .17% P level was adequate. This result was in agreement with that of Burroughs et al. (1956) and indicated that the P requirement for weight gain and feed response is greater than for bone growth or maintenance of plasma inorganic P level. Pope et al. (1958) could not show improved performance in finishing steers fed milo-sorghum silage rations when the P level of the ration dry matter was increased from .23 to either .32 or .41%.

A dietary Ca:P ratio between 1:1 and 2:1 is assumed to be ideal for growth and bone formation since this approximates the ratio of the two minerals in bone. Ruminants can tolerate a wide range of Ca:P ratios, especially when vitamin D levels are high (Underwood, 1981). In a trial with sheep, Young et al. (1966) fed a diet containing .26% P with a total Ca:P ratio of 10:1, which had no

effect on P absorption. Wise et al. (1963), in a factorial experiment, fed calves three levels of dietary Ca (.27, .81 and 2.43%) and three levels of dietary P (.17, .34 and .68%), yielding nine Ca:P ratios ranging from .4:1 to 14.3:1. All ratios between 1:1 and 7:1 gave satisfactory results in regards to growth and feed efficiency; however, ratios below 1:1 and above 7:1 caused significant decreases in the above parameters. Similar results have been reported by Call et al. (1978) when beef heifers fed for 2 yr on diets containing Ca:P ratios of 7:1 to 9:1 had no adverse effects on growth, feed efficiency or fertility. Results with Holstein steers which were fed Ca:P ratios of 1:1, 4:1 and 8:1 (Ricketts et al., 1970) indicated that gain and growth were less than the other groups when an 8:1 ratio was fed.

When assessing the effects of Ca:P ratios, it is imperative that one take into account the absolute levels of these minerals. When either level is deficient, increasing the level of the other can result in deleterious effects upon the animal. Although the number of studies with cattle are limited, it is believed that a ratio in which P exceeds Ca can be quite detrimental when levels of these minerals are marginal (Wise et al., 1963; Miller, 1979; ARC, 1980). This last consideration in lieu of absolute Ca and P intakes becomes critical when researchers are looking for a positive response to P supplementation. High Ca intakes may depress $1,25(\text{OH})_2\text{D}_3$ production with a subsequent decrease in P absorption, PTH secretion, salivary P concentrations and mobilization of P from bone. Under these conditions with a low P intake, a growth response to P

supplementation could occur, provided no other nutrients are limiting. If dietary Ca intake was low, the converse of the aforementioned situation would occur, and possibly more P would be available in tissue for growth. Therefore, under these conditions, a growth response to P supplementation may not occur.

One must, therefore, keep in mind when assessing the P requirement of various classes of beef cattle if all other nutrients are in an adequate and available supply to meet the animals needs for a given level of production. The tendency for seasonal fluctuations of N and P levels to parallel each other in pastures has been observed worldwide (Hemingway, 1967). From such considerations, Eckles et al. (1935) concluded that the P deficiency occurring in the field was a combined deficiency of N and P, rather than a P deficiency alone. Du Toit et al. (1940, cited by Little, 1970) also considered that the low protein content of pastures over much of the year was the major problem and felt that the mobilization of skeletal reserves masked appearance of any specific clinical deficiencies. Becker et al. (1933) also reported similar findings (i.e., low P content of pasture conjoined with low crude protein content of the same pastures). Cohen (1974) concluded that P supplementation (regardless of season) would not improve liveweight performance when beef cattle graze pastures of low quality (i.e., low N, low energy or limiting in other nutrients).

Little (1980) in a pair feeding regimen fed seven Hereford and one Brahman cross steer and five Brahman cross heifers (average initial weight 165 Kg) an ad libitum basal diet of *Stylosanthes*

humilis (1.8% N, .12% P and .77% Ca, DMB), with or without P supplement at the rate of $5 \text{ g} \cdot \text{d}^{-1}$ (provided as NaH_2PO_4 three times per wk) for 70 d. Irrespective of treatment, feed was consumed in adequate amounts to support $.5 \text{ Kg} \cdot \text{d}^{-1}$ gain. No differences were found between groups with respect to voluntary feed intake (even when expressed on a metabolic body size). Rib biopsy P concentration (expressed as $\text{mg P} \cdot \text{cm}^{-3}$, fresh basis) and plasma inorganic P concentration yielded no differences between groups. The author thus concluded that the unsupplemented animals were not P-deficient and that the .12% P (DMB) was adequate for this level of production (i.e., for .5 Kg gain per day). This was in agreement with earlier work (Little, 1968) in which 16 yearling Droughtmaster cattle (eight heifers, eight steers) were fed a basal diet of *S. humilis* which contained .07% P (DMB) but adequate in N and energy. Phosphorus was added at the rate of 0, 4, 8 or $12 \text{ g} \cdot \text{d}^{-1}$ as potassium phosphate. The unsupplemented P level was inadequate for growth compared to the other groups. Little (1968) indicated that slightly less than .13% P (DMB) would be adequate for the rate of gain obtained ($.5 \text{ Kg} \cdot \text{d}^{-1}$) as there were no significant differences among gains made in the supplemented groups.

In a similar study, Call et al. (1978) investigated the influence of P on growth and reproduction in beef heifers. In this study, two replicates of 48 Hereford heifers (initially 7 mo age and 165 Kg) were individually fed a basal ration (pelleted or chopped alfalfa, chopped wheat straw and grass hay, top-dressed with beet molasses) containing either .14% P (as-fed) or the basal ration top

dressed with sufficient monosodium phosphate to provide .36% P (as-fed). Rations were adequate in protein, energy, minerals and vitamins. Animals were fed ad libitum. The Ca:P ratio ranged from 3.5:1 to 7.1:1 for the high P and low P groups, respectively. The experiment lasted approximately 2 yr. There was no difference between groups in average daily gain (ADG); .44 vs .45 Kg·d⁻¹ for the .14 and .36% P groups, respectively; feed efficiency was also similar in the two groups. Also the authors reported no signs of depraved appetite or other clinical signs of P deficiency. These results were similar to those of Little (1980) and Wise et al. (1963); however, these results are in disagreement with the findings of Teh et al. (1982) who reported increased gains in dairy calves fed .31 vs .24% P (.84 vs .63 Kg·d⁻¹, respectively).

In a follow-up study, Butcher et al. (1979) continued feeding the animals reported on by Call et al. (1978) for an additional 2 yr period. After 4 yr on the same diets, Butcher et al. (1979) reported no significant differences between the .14 and .36% P groups with respect to growth rate or voluntary intake. Numerical differences in blood, bone and muscle tissue were seen; however, these were not deemed as physiologically significant. After 4 yr, one-half the animals on both the .14 and .36% P groups were reassigned to a .09% P level. Appetite was somewhat depressed after 8 mo on the .09% P diet, whereas, after 14 mo, one-half of the cows on this reduced level showed reduced intake. Body weight changes were not different between the .14 and .36% P groups at this time. At necropsy, the

cows from the .09% P group also showed reduced bone density and increased likelihood of fractures.

Reproduction

The relationship between the nutrition of animals and their fertility has offered researchers some of the most interesting and important problems in animal production over the years. Evidence (either circumstantial, practical or research in nature) has demonstrated that dietary deficiencies may exert an adverse effect upon one or more of the several stages in the reproductive cycle, with the general observation that fertility and reproduction exhibit abnormalities ranging from slight repression to complete cessation--sometimes with the sudden interruption of pregnancy itself.

With beef cattle, breeding, pregnancy and lactation for the most part take place on feeding regimes that primarily utilize pasture, range or some type of preserved forage. It was under these types of conditions that P was initially shown to be important in the nutrition of cattle and sheep. Many studies have indicated inadequate P levels in range and pasture, especially during the late winter months. Phosphorus deficiency of grazing ruminants has been reported in many areas of the world, including the United States, South Africa and Australia, under similar conditions.

In many studies where P supplementation on pasture or range has proven beneficial, the inability to state the P intake from the forage has been a major problem in studying the minimum P requirements for pregnancy and lactation. Nonetheless, P supplementation has been both beneficial and essential, especially

during the fall and winter periods (Black et al., 1949; Thomas and VanHorn, 1967; Little, 1975; Loxton et al., 1983). Also, it is not always clear from the literature whether adequate levels of all nutrients other than P were available, when a so-called P deficiency is reported.

Theiler et al. (1928), in an early study with 200 breeding cattle on the P deficient ranges of South Africa, attributed the strikingly subnormal calf crop of a control herd (51%) not receiving bonemeal in comparison with that of a bonemeal fed herd (80%) to irregular breeding brought on by the drain of milk secretion on the P reserves of the body. Subnormal fertility was associated with depressed or irregular estrus, thus preventing or delaying conception. The calves from cows receiving the supplement at 15 mo of age weighed 318 Kg, nearly twice that of calves born to the control cows. However, it may be unwise to attribute all the benefit from bonemeal supplementation to P due to its high protein content (Cohen, 1975).

Eckles et al. (1926) observed a marked inhibition of estrus in cattle showing the general signs of a P deficiency. Out of 14 cows studied from a P deficient area, all of which had been in pasture with a bull, two were with calf and seven had nonfunctioning ovaries. After a period of 3 to 9 mo, all animals eventually showed estrus. Exact cause of the inhibition of estrus, however, could not be directly stated. Huffman et al. (1933) fed either .22 or .44% P (DMB) to dairy heifers from 3 mo of age to first calving. The .22% ration resulted in lower plasma inorganic P concentration, decreased

appetite and weight gain, but seemed to have no adverse effects in regards to reproduction in these animals.

Eckles et al. (1935) in a critical study pertaining to an uncomplicated P deficiency on various parameters associated with estrous cycle and reproduction in dairy cows concluded that P deficiency did not cause abnormal estrous cycles although it appeared to reduce "breeding efficiency" (this, however, was complicated by the incidence of brucellosis). These authors postulated that the disturbances in estrus and the low calf crop of cattle in P deficient areas, under natural conditions, were probably due to multiple nutrient deficiencies.

This is in agreement with Guilbert and Goss (1932) who noted both cessation as well as long or irregular estrous cycles of rats fed low dietary protein levels (i.e., 3.5 to 5.0%). Grossly P deficient rations fed to heifers ($3 \text{ g P} \cdot \text{d}^{-1}$) did not affect estrus or normal ovulations when fed for an extended period (Theiler et al., 1937). Palmer et al. (1941) studied combined P and protein deficiency, comparable to the problem seen under field conditions. They observed irregularities in estrous (such that periods of estrus appeared to be missed), but no adverse effect on cyclical ovulation or conception. Some evidence of delayed puberty (average age at first ovulation and estrus was 14.5 mo) and dystocia were observed. However, heifers on a diet specifically deficient in P continued to exhibit estrus normally.

Knox and Watkins (1942) reported that 42% of the range grass samples collected throughout the year in New Mexico were deficient in

P, and 75% of those collected in late winter were deficient. These authors found the use of a P and Ca supplement, such as bonemeal, resulted in reduced death losses in newborn calves, larger numbers of calves weaned and greater weaning weight of calves. Black et al. (1943) reported only 64% of control cows produced calves on range in southern Texas, compared to 85% in cows given supplemental P (as either bonemeal or disodium phosphate); only slightly more than 30% of control cows calved in two consecutive years, whereas 75% of the supplemented cows had calved. Practically all of the control cows were observed chewing bones, which did not occur in supplemented cows. Supplemental P had no influence on birth weight of calves; however, weaning weight per calf was 31.4 Kg more in the supplemented vs cows.

Hignett (1950) observed anestrus with low P levels, whereas silent and irregular estrus periods were associated with moderate deficiencies and infertility with marginal levels. These same authors (Hignett and Hignett, 1951) in a survey of 50 dairy farms reported that conception rate was improved in cows fed rations with increasing P content. They suggested for cows producing milk (4.5 to 6.8 Kg daily, which is common in beef cows) a requirement of about $30 \text{ g P} \cdot \text{d}^{-1}$. Hignett and Hignett (1952) claimed that an intake of at least $6.6 \text{ g P} \cdot 100 \text{ Kg}^{-1}$ body weight was required for "reasonable" conception rates in heifers and that a P deficiency decreased estrus activity. Subsequently, however, Littlejohn and Lewis (1960) obtained no difference in fertility between animals given 2.0 and $11.4 \text{ g P} \cdot 100 \text{ Kg}^{-1}$ body weight 2 mo prior to breeding.

O'Moore (1952) reported cattle herds with a history of infertility were always associated with less than .15% P in the dry matter of pastures. When sodium phosphate was supplemented with or without protein, only those that received the combination exhibited estrus during the feeding period. In contrast to this, Bisschop (1964) reported that the highest level of P in South African pastures in 23 yr of monthly sampling was .13%. Bisschop (1964) concluded that at no time did the natural pasture provide sufficient P or crude protein for optimum production; however, both dairy and beef type cows were shown to have grown and reproduced under these conditions, although rather inefficiently. Ward (1968) and Lamond (1970) reported the effects of undernutrition on fertility from Zimbabwe and northern Australia, respectively. Lactating cows, with few exceptions, were not calving 2 yr in succession. In P deficient areas, if a calf is produced, it was observed that cows may not come into a regular estrus again until body P levels were restored, either by supplementing P or by ending lactation.

Over a 5 yr period on the King Ranch in southern Texas (Black et al., 1949), the percent calf crop decreased in control cows from 91 to 22%, whereas P supplemented cows maintained a fairly constant calving percentage. Nelson et al. (1951) reported that heifers consuming grass, hay and supplement providing 10 g P (total) daily was adequate to meet the P requirement for reproduction and lactation; however, less than this was inadequate. In Montana (Thomas and Van Horn, 1967) heifers receiving increasing levels of P supplementation in addition to winter range had a higher percentage

calf crop over a 5 yr period. Little (1975) found a shortened interval between calving and first estrus (62.4 vs 74.9 d) and increased proportion of cows in estrus by 12 wk after calving (70 vs 50%) for P and protein supplemented and unsupplemented cows, respectively. McDowell (1985) in a review reported that the average of 16 cattle reports from tropical countries on the effect of P supplementation on calving percentage was 75.6% for those receiving a P supplement versus 52.9% for controls.

Holzschuh et al. (1971) fed yearling heifers either 7.8 or 12.7 g P d⁻¹ for 21 mo. They reported that the heifers did not differ in age at first estrus, period between first insemination and conception, proportion which became pregnant or duration of pregnancy. These authors emphasized that the heifers became pregnant despite P deficiency. Call et al. (1974) fed heifers rations containing either .16 or .51% P (DMB) during the growing and breeding period. The authors found no differences in weight gain or conception rate between the groups. No adverse effects on estrus or conception rate in dairy heifers fed .21 to .24% P led Noller et al. (1977) to conclude that these levels were sufficient for reproduction. Call et al. (1978), in a study with 96 Hereford heifers that were fed a basal ration containing .14% or the basal ration supplemented with monosodium phosphate to provide .36% P (air-dry basis) for 2 yr, reported that the lower level was adequate for growth, puberty, conception, gestation and lactation. In this study, there was no difference in age at puberty between groups; the heifers that received the .14% P ration had a 96% pregnancy rate with 91%

live calves compared to 100 and 93%, respectively, for the supplemented heifers (these differences were not significant). Butcher et al. (1979) reported on results from the study conducted by Call et al. (1978). The 1979 report revealed no reproductive differences between the .14 and .36% P groups through three gestations. After this time (i.e., 4 yr on trial), one-half the animals in each group was randomly assigned to a .09% P group. Cows that were placed on the reduced P level (.09%) went through the last two-thirds of pregnancy, lactation and rebreeding with no apparent adverse effects. All cows were bred by natural service. Conception rates of 60, 91 and 95% were reported for the .36, .14 and .09% P groups, respectively. These data would indicate that P levels as low as .09% are still adequate for maintaining fertility in mature beef cows. Call et al. (1982), reporting on the same animals through their eighth gestation, disclosed that cows on the .09% P ration for an additional year developed a complex syndrome which included the following: unthriftiness, body weight loss, reduced feed consumption, lameness and impaired reproductive performance (i.e., cows failed to rebreed after sixth calving). When these cows were subsequently changed to a diet containing .14% P, voluntary intake increased, the animals developed a glossy hair coat and all were bred within their first two cycles after bulls were introduced. These observations were indicative not only of the insidious nature of P deficiency in cattle, but also the great adaptive power of the animal to a wide range of P intakes (accomplished mainly through renal and salivary P conservation mechanisms and bone mobilization).

Hurley et al. (1982) conducted an experiment to study the effects of varying P levels (from 73 to 246% of NRC recommendations) on estrous cycle and endocrine status of dairy heifers and reported that dietary P inadequacy did not alter reproductive endocrine function or estrus intensity in heifers in this trial. No difference in blood serum concentration of progesterone, estradiol or luteinizing hormone was noted around the time of estrus in the heifers.

Judkins et al. (1985) reported on a P supplementation trial involving 78 Angus X Hereford range cows (39 control cows received salt only, 39 cows received a free choice mineral supplement with P) over a 5 yr period in New Mexico. These authors reported that fertility was not altered by P supplementation. However, no data were reported on forage intake or P content of forage available.

Greene et al. (1985) conducted an experiment to study the effects of either low P (.04%) or adequate P (.44%) on growth and estrous activity of rats. These authors concluded that the effect of a P deficiency during gestation, lactation or weaning in rats was limited to pup growth and survival postpartum and did not appear to be associated with the regularity of estrous cycles, conception rate or embryo survival.

In a recent study, Call et al. (1987) reported on the clinical effects of low dietary P fed to 34 lactating Holstein dairy cows. Three dietary concentrations of P were fed (.24, .32 and .42%, DMB) for up to 12 mo. These authors reported that the reproductive performance in the .24%P group was superior to both other treatment

groups. Cows receiving .24%P in the diet achieved a 92% pregnancy rate as compared to 87 and 76% for the .32 and .42%P groups, respectively. Cows on the lower P diet also required fewer breedings per pregnancy. Also, cows receiving the .24%P diet tended to resume cycling of estrus earlier after calving was completed than did cows in other treatment groups.

Based on published results in the literature, there appears to be wide variation in the definition of minimum P requirements for the various phases of reproduction in female beef cattle. Phosphorus deficiency is accepted as a major nutritional factor depressing reproductive efficiency in mammals. In some studies, ovarian function and fertility of females appear to be quite sensitive to P intake; however, other work has failed to show a diminished reproductive performance of animals on P deficient diets. It must also be stated again that many times P deficient diets are usually low in protein and energy, which along with lowered feed intake, generates a P, energy and protein deficiency. Therefore, it may be suggested from some research that a P deficiency, per se, will not affect reproductive performance until malnutrition occurs. Also, although reproductive failures have been reported when animals consume diets low in P, the precise physiological mechanisms involved have not been identified.

Assessment of Phosphorus Status

Problems of P deficiency in cattle are widespread (Mitchell, 1947; Underwood, 1981; McDowell, 1985), yet there is no one

completely satisfactory method of objectively assessing the P status of the bovine. Because the problem of P deficiency is so prevalent, it is imperative to identify the most precise yet practical indicator of P status in cattle.

The detection of mineral element deficiencies or excesses involves clinical, pathological and analytical criteria as well as response from specific element supplementation. Clinical signs of mineral deficiencies along with soil, water, plant and animal tissue analyses have all been used with varying degrees of success to establish mineral deficiencies and toxicities (McDowell, 1985).

A goal of mineral nutritionists for many years has been the development of simple and accurate biochemical measurements of the status of animals for the minerals in which there are important practical problems (Miller and Stake, 1974). When the evidence from clinical, pathological and biochemical examinations of the animal and from chemical analysis of the diet and its components is combined and assessed, it is usually possible to detect and define any nutritional abnormality of mineral origin, even when it is mild (Underwood, 1981).

Specific clinical signs like aphosphorosis may be useful in diagnosing a mineral deficiency, although by the time these signs are apparent, the deficiency is usually in an advanced state (Little, 1982). McDowell et al. (1984) have reported on the essentiality of chemical analyses and biological assays when studying mineral imbalances (particularly the borderline or subclinical cases which may not produce signs specific to a single mineral).

Various criteria have been used as diagnostic aids in assessing the P status of grazing ruminants: feed samples (both hand plucked grab samples and samples collected from esophageally fistulated animals), soil analyses, depraved appetite, bone composition, bone growth, bone breaking strength, blood analyses, rumen contents, rumen fluid, fecal P concentrations, salivary P concentrations, hair P concentrations, P concentrations in milk, P balance studies, muscle P concentration, analysis of body organs and the performance of animals in terms of growth and reproductive efficiency. Many of these will be reviewed in the following sections.

Feed

Forage, according to the Netherlands Committee on Mineral Nutrition (NCMN, 1973), is the most suitable sample for diagnosing a P deficiency. Under controlled feeding experiments where animals have no access to any feed other than what is offered, this may be a useful tool. However, it is a more complicated situation in the pasture with the grazing ruminant due to their selective grazing behavior. Selectivity of the grazer may vary with animal species, availability of plant, stage of maturity, intensity of grazing and weather condition (Cook, 1964). Hand plucked pasture samples may not represent the diet selected by the animal. Other problems associated with this type of sampling in assessing mineral adequacy is the difficulty of estimating forage intake and digestibility. The use of esophageally fistulated animals on pasture has made this procedure far more reliable; however, salivary P contamination makes these samples unsuitable for estimating dietary P intake levels (Langlands,

1966; Little, 1972a) unless salivary P is labelled with ^{32}P prior to collection (Little et al., 1977).

Blood

Whole blood or blood serum or plasma is widely used for studies in mineral nutrition (McDowell, 1985) and since it is easily obtained from live animals, it is an ideal tissue for study. However, according to Underwood (1981) values significantly and consistently above or below so-called normal concentration or ranges provide suggestive but not conclusive evidence of a dietary excess or deficiency of particular minerals.

The first known response to a dietary deficiency of P is a fall in the inorganic P fraction of blood plasma (Underwood, 1981). Changes in blood inorganic P can occur quite fast after changing animals to P deficient rations. Henderson and Weakly (1930) noted a change from 8.0 to 4.6 mg P·dl⁻¹ plasma within 4 d after a ration change from an adequate to an inadequate P intake. Several authors have reported increases in the blood inorganic P concentration after "adjustment" to a ration low in P. Bone P is mobilized to maintain a normal blood inorganic P level in P deficient animals but this mobilization is not rapid (Duncan, 1958); therefore, blood may only reflect rapid changes in dietary P whereas P deficiency, caused by prolonged low levels of dietary P, may be better detected in bone.

The usefulness of inorganic P in blood serum or plasma is still a matter of contention in regards to it being an adequate criterion for assessing P status in ruminants. The NCMN (1973) reported that blood is most relevant for detecting only Zn deficiency, whereas, Ca

concentration in blood plasma is influenced only by severe deficiency and that of inorganic P cannot be used as a practical criterion at all due to its great variation and the poor understanding of the factors that cause this variation. Underwood (1966), conversely, indicated that serum inorganic P was a satisfactory criterion for assessing the P status of ruminant animals. Conrad (1978) has also suggested that most research groups consider serum inorganic P to be sufficiently sensitive and recommend it for diagnosing P deficiencies, while the NCMN (1973) as previously mentioned recommends forage (i.e., feed) analysis.

Many factors have been identified in influencing blood serum or plasma inorganic P levels. Irving (1973) reported that the P level in blood is not nearly as constant as the Ca level. Exercise (Palmer et al., 1930; Gartner et al., 1965) and excitement (Eckles et al., 1932) in cattle prior to bleeding have been reported to increase blood inorganic P concentrations. Burdin and Howard (1963) have reported that increased storage time and/or temperature postsampling increased blood P due to hydrolysis of organically bound P from phosphoric acid esters such as ATP. An increase of 64% in organic P during 1 wk storage at ambient temperature occurred compared to only a 4% increase when blood was stored at 4 C for 12 d. The type of protein precipitant in preparing the sample for analysis (Little et al., 1971) and water restriction (Rollinson and Bredon, 1960) have also been shown to increase blood P levels. Phosphorus values are also affected by parasites. Blood P increased approximately 2-fold

in liver fluke-infested White Fulani cattle in Nigeria (Ogunrinade et al., 1980, cited by Mtimuni, 1982).

Recently, Perge et al. (1983) indicated that the effect of feed on Ca and P content in blood serum and saliva of wethers is influenced not only by dietary supplies (i.e., amounts of the elements in the ration) but also by the time of sampling. As Forar et al. (1982) have pointed out, time of sampling should be standardized when collecting blood samples to reduce the effect of diurnal variation in plasma inorganic P concentration in cattle. Wise et al. (1963) also have reported rapid increases in blood P content after feeding, which could lead to false readings.

Inorganic P concentrations in plasma differed between animals in a herd and between months within animals (Blooser et al., 1951). Wilson et al. (1977) reported a tendency for inorganic P concentration in plasma to decrease prior to parturition, with the lowest point at the time of calving. Blood plasma-mineral profiles of lactating dairy cows showed a decrease in P concentration at parturition, except in young cows fed plain salt versus those receiving a mineral supplement (McAdam and O'Dell, 1982). Only minor fluctuations were observed in P concentration for animals on each treatment throughout lactation.

Reference has already been made to studies where blood inorganic P level was used to study P adequacy. Theiler et al. (1937) stated that blood inorganic P may drop to one-fourth of the normal value even before deficiency signs can be observed. Murray et al. (1936) suggested that blood inorganic P levels indicating P deficiency are

less than $3.8 \text{ mg P} \cdot \text{dl}^{-1}$ serum in mature cattle and $5.3 \text{ mg P} \cdot \text{dl}^{-1}$ serum in young cattle. Cunha et al. (1964) considered cows with concentrations lower than $5 \text{ mg P} \cdot \text{dl}^{-1}$ serum as deficient. Thompson (1978) indicated that, usually, the first evidence of P deficiency is a drop in plasma inorganic P below the normal values of $4\text{--}6 \text{ mg P} \cdot \text{dl}^{-1}$ of plasma for adults and $6\text{--}8 \text{ mg P} \cdot \text{dl}^{-1}$ of plasma for young animals.

Becker et al. (1933), in Florida, reported that nursing cows grazing P deficient pastures had an average of $2.55 \text{ mg P} \cdot \text{dl}^{-1}$ of serum, whereas these same cows when supplemented with bone meal had values of $4.02 \text{ mg P} \cdot \text{dl}^{-1}$ of serum. In growing-finishing steers (Beeson et al., 1941), plasma inorganic P was directly correlated with P level in the ration with maximum gain being observed on .21% P (DMB) which resulted in a plasma inorganic level of $6.6 \text{ mg P} \cdot \text{dl}^{-1}$ plasma; controls fed a ration of .13% P (DMB) had a plasma inorganic P level of $3.6 \text{ mg P} \cdot \text{dl}^{-1}$ plasma. In agreement with this study, Cohen (1974) reported that blood plasma P concentration in cattle was significantly related to P intake, but that the relationship varied depending on the time of day at which the samples were collected. Normal values for plasma inorganic P are $4\text{--}6 \text{ mg P} \cdot \text{dl}^{-1}$ for adults and somewhat higher, often $6\text{--}8 \text{ mg} \cdot \text{dl}^{-1}$, for very young animals (Underwood, 1981).

Kiatoko et al. (1982) in a study of the mineral status of beef cattle herds from four soil order regions of Florida found that mean plasma P in all regions was above $4.5 \text{ mg P} \cdot \text{dl}^{-1}$ during the wet season, while during the dry season, concentrations in the Histosol soil order were below this level. Of all the animals studied, 13%

had low plasma P during the dry season. Read et al. (1986a) recently concluded that low plasma P levels adequately reflected low P intakes; however, plasma P concentration was a poor indicator when higher levels of P intake are concerned.

With regards to usefulness of whole blood as a criterion in assessing P status in the bovine, the literature is limited. There appears to be great variation in what is the accepted normal range of values. Rodgers (1975) reported normal whole blood P values in cattle of 35 to 45 mg P·dl⁻¹; whereas, Call et al. (1978) have reported values of 18 to 21 mg P·dl⁻¹ in heifers fed .36% P (as-fed). Perhaps a great deal of this variation is a product of the method used in preparing the whole blood sample for P analysis. Many of the factors affecting either serum or plasma inorganic P level, such as water restriction and parasite load which would affect the packed cell volume also greatly influence whole blood P concentration. It should also be considered that whole blood P levels are a combination of inorganic P and significant amounts of organically bound P, the latter which is not "available" to the animal as such. Miller (1983) has stated that total plasma P (i.e., includes plasma proteins) is not a very sensitive measure of P deficiency for this very reason.

Hair

Hair has many innate properties that make it a likely tissue for biopsy; it is easily collected with little trauma to the animal and can be stored until analysis is convenient because it does not deteriorate rapidly (Combs et al., 1982). For several years, it has

been proposed that body stores of minerals could possibly be estimated from hair analyses, as growing hair is metabolically active and a sequestering tissue. Hair may, therefore, mirror concentrations of minerals that were in the hair follicle at the time the hair was formed. However, mineral content of hair may also reflect surface contamination by minerals in urine, feces, sweat, feed and air-borne matter (Combs et al., 1982). Also, it has been shown that the mineral content of hair is affected by season, breed, hair color within and between breeds, sire, age and body location (Miller et al., 1965; O'Mary, 1969; 1970; Hambidge et al., 1972; Kiatoko et al., 1982).

The use of hair analyses to monitor the intake of Ca and P has for the most part been unsuccessful. Anke (1966) reported that dietary supplementation with Ca and P significantly increased concentrations of Ca and P in pigmented hair of dairy cattle. A positive correlation (Anke, 1967; cited in Combs et al., 1982) was obtained between P content of feed and that of black hair of cattle. In the latter study, Anke (1967; cited in Combs et al., 1982) concluded that the supply of P to cattle was well reflected in hair P content. This finding is in agreement with Legel (1971) who reported that hair was suitable as an indicator of P status at certain times of the year. These authors reported that P content of hair showed seasonal variations which were greater when P intake was low (7.8 vs 12.7 g P·d⁻¹) in this trial. The P concentration of pigmented hair had fallen significantly after 8 wk on the low P diet. In contrast, Wysocki and Klett (1971) reported low

correlations between Ca and P intake in ponies and the Ca and P content of their hair. Cohen (1973a) sampled 15 Angus and Angus X Hereford yearling steers five times from December, 1969, to December, 1970 (3 mo apart). He found no difference in P content of hair between sampling times and no significant relation between P content of pasture and hair. This is in contrast to Kiatoko et al. (1982) who reported higher P concentrations in dry versus wet season (219 vs 104 ppm, respectively). Cohen (1973b) found that drenching growing steers with either 35 or 70 g P (given six times per wk as sodium dihydrogen orthophosphate) did not change hair P or Ca concentrations. From his two studies, Cohen (1973a, b) concluded that hair P was not a satisfactory indicator of P status in cattle.

Bone

General characteristics. Bone is a highly specialized connective tissue that consists of cells embedded within a gel-like substance that becomes mineralized to varying degrees. The method of secretion and the manner in which the cells become embedded in the matrix are similar to those phenomena observed in cartilage. Although the fibrous and ground substance components of bone are similar to those of cartilage (collagen and acid mucopolysaccharides), the mineral portion of the matrix makes bone different from other connective tissues (Banks, 1981). This mineral fraction consists of amorphous calcium phosphate and the hydroxyapatite crystal.

Cortical bone is composed of approximately 69% inorganic matter, 22% organic matter, and 9% water (Urist, 1980). Over 99% of the

body's Ca and 80% of the P are found in bone and, in conjunction, these minerals comprise the major portion of the inorganic fraction as hydroxyapatite. Initially, unstable forms of amorphous calcium phosphate are formed, presumably because hydroxyapatite itself cannot be formed from its constituent ions due to its large size and crystallographic structure. As conditions change (particularly pH), other forms are precipitated until hydroxyapatite is formed. Generally, more recent precipitates have a low Ca:P ratio of 1 to 1.5:1, while aged precipitates have a ratio of 1.4 to 2:1. Magnesium, Na, K, F, Sr, citrate and carbonate ions are also present among bone salts. It is believed that these are adsorbed to hydroxyapatite crystals, rather than forming crystals of their own. It is the mineral fraction of bone which contributes to the great compressional strength of bone (Guyton, 1981).

Collagen comprises 90% of the organic fraction of bone. The remaining noncollagenous portion consists of other proteins, proteoglycans and lipids. Collagen is the major structural protein and displays great tensile strength, requiring a load of 10 to 40 Kg to break a fiber 1 mm in diameter (Urist, 1980).

Although the exact mechanism(s) of bone calcification is not completely understood, it is believed that certain inhibitors, for example, pyrophosphates, are present initially. When this inhibitor is removed, crystallization is initiated. Collagen fibers have an affinity for Ca salts and there are nucleation sites along the molecule which encourage formation of a crystal seed and then further precipitation of the salts. Although many other theories on the

mechanism of calcification exist, this nucleation theory is the best attempt at explanation of the bone mineralization process to date.

Contrary to perceptions that might be formed from handling a dried portion of the skeleton, bone is not a "dead" static tissue, but rather a dynamic, metabolically active tissue in a constant state of change. The extensive blood supply to bone gives some insight to its viability and metabolic activity. Wilson (1972) reported that every gram of bone has an exposed surface of several square meters providing it with tremendous surface area for vascular exchange. The large surface area is important to both Ca and P metabolism. Through storage, resorption and mobilization of these elements, bone is responsible for the long term homeostasis of these minerals.

Bone consists of three types of osteogenic cells: 1) osteoblasts, 2) osteoclasts, and 3) osteocytes. The bone cells are responsible for both structural and metabolic functions of bone. Osteoblasts (bone forming cells) secrete collagen and ground substance and lie on the surface of bone. Osteoclasts (which are multi-nucleated) are responsible for resorption of bone. Generally, osteoclasts resorb bone from fully mineralized tissue rather than from osteoid surfaces. An adaptive modification in the plasma membrane, the brush border, is thought to be capable of removal of bone mineral as well as digestion of the organic matrix (Banks, 1981). Osteocytes are osteoblasts that have become embedded in bone. Microscopically osteocytes are similar to osteoblasts. They continue the same osteoblastic activity of maintaining the matrix

only to a lesser degree (Ham and Cormack, 1979). Osteocytes may also possibly function in the resorption of bone.

Osteogenic cells are usually found on the endosteal and periosteal surfaces of compact bone (Vaughan, 1970). Bands of lamellar bone are adjacent to these layers, which contain osteocytes and Haversian canals. These canals, which are lined with osteogenic cells, are the pathways for blood vessels supplying bone. They predominate internal to the lamellar bone. Each Haversian system is surrounded by osteocytes communicating with each other through their canaliculi. A cement line separates each Haversian canal from other canaliculi, across which there is no communication. The area around each canal bounded by a cement line is known as an osteon, or resorption cavity. Some osteons show no resorption-apposition activity for some time, while others show varying amounts of remodeling. Lacunae are the areas of mineralized matrix left by some earlier remodeling involving Haversian canals or lamellar bone. Trabecular bone contains fewer Haversian systems and the remodeling, which is more active than that in compact bone, takes place on the surface of the trabeculae. Growth in length occurs by endochondral ossification, which takes place at the epiphysis, while growth in diameter occurs by concurrent resorption on the endosteal portion of the bone and apposition on the periosteal side.

The turnover of bone is important to the performance of its structural and metabolic functions. Mature bone is constantly being resorbed and reformed. The normal process of internal remodeling may occur with no change in the gross anatomic structure of the bone.

Banks (1981) has reported that internal remodeling may occur in response to altered vascular relationships that require the repositioning of vessels within compact bone for adequate osteocyte nutrition, biomechanical stress, dietary deficiencies, immobilization and/or disuse, and altered endocrine function.

Trabecular bone is more labile than compact bone. Trabecular remodeling activity may be as great as three times higher than compact bone. Remodeling though not a rapid process is dynamic. Banks (1981) has reported the time required from resorption to reformation of a mature Haversian system may require months. For maintenance of normal skeletal volume, resorptive and formative processes must be in balance. Homeostatic control of remodeling is achieved by balanced activity of osteoblasts and osteoclasts.

Wolff's Law states that remodeling takes place so that skeletal form and mass adjust to provide optimum bone strength (Lanyon, 1982). Frost (1973), building on Wolff's Law of structural remodeling, developed the Flexure Drift Law which stated that during bone formation, lamella orient parallel to the direction or resultant of the greatest tension and/or compression stress. Frost (1973) also proposed that this force response characteristic may account for the anatomic conformation of various bones.

Techniques used in assessing phosphorus status. Modern animal production practices have greatly enhanced the importance of optimum bone development and strength at an early age, especially in regards to prolonging the useful life of breeding animals. All phases of livestock production have realized the need for quality bone

development. This is clearly seen where the push for maximum production in beef, dairy, poultry and swine operations under confinement situations has created need for situations where optimal bone strength is important in relation to the performance of the animal.

(1) Rib and tail biopsies. Withdrawal of minerals during periods of inadequate intake does not take place equally from different parts of the skeleton. The spongy bones, ribs, vertebrae and sternum, which contain less inorganic salts, are the first to be affected, together with the cancellous ends of the long bones. The compact shafts of the long bones such as the humerus, femur and tibia and of the small bones of the extremities are the last reserves to be used. In each case, the essential change is a reduction in the total mineral content of the bones, with little alteration in the proportions of the minerals in the remaining ash (Underwood, 1981). This is probably due in fact to the mechanism by which mineral is lost from bone (i.e., resorption versus demineralization). Resorption involves the removal of both bone matrix and mineral matter, consequently reducing bone mass and ash, but not necessarily bone ash percentage. Duncan (1958) stated that percentage ash in a sample of bone gave no indication of the status of reserve and suggested that comparison of the ash weight of representative whole bones would be a better indication. Maynard et al. (1979) reported that in mammals the bone is made of approximately 36% Ca, 17% P and .8% Mg, based on dry fat-free bone. Benzie et al. (1959) reported that the axial skeleton and ribs of the ewe were resorbed and

rehabilitated more readily than long bones, therefore suggesting changes in nutrient intake is better detected from the axial skeleton and ribs.

Most studies of the skeleton were conducted on animals slaughtered specifically for that purpose, until the development by Little (1972b) of a rib bone biopsy technique which enables serial sampling of both cattle and sheep. This technique has many advantages in studies where the monitoring of Ca and P in the skeleton are primary goals--especially in terms of economics and the ability to sample the same animal repeatedly.

Little (1972b) reported that expressing Ca and P content per unit volume has greater sensitivity than per unit of fresh weight, whereas expressing Ca and P on a moisture-free, fat-free basis was not sensitive because changes in water and fat contents of bone were not taken into account. In a 6 wk depletion study of yearling cattle (diet contained .08% P and 8% CP, DMB), Little (1972b) revealed an increase of 23.0 to 27.9% in moisture and 1.7 to 5.2% in fat of rib bone biopsies taken at the initiation of depletion and the end of the 6 wk period, respectively. Rib bone biopsies also indicated a decreased bone density (as measured by specific gravity), 1.68 vs 1.59 g·cm³ for pre- and postdepletion periods, respectively.

Little and Minson (1977) have suggested that the same rib should be sampled in all animals in any one investigation due to variation of P content among ribs within an animal. They suggest the use of the 12th rib for best results. Also, Little and Ratcliff (1979) reported that unbiased results may be obtained from samples of bone

taken from a rib sampled previously, 6 mo or more after the earlier biopsy, and that it is advisable to obtain the first sample from a site ventral to that proposed for the second sample. It has been suggested by Little and Shaw (1979) that possible critical levels of $120 \text{ mg P} \cdot \text{cm}^{-3}$ bone from the 12th rib may indicate deficiency of P while levels over $150 \text{ mg P} \cdot \text{cm}^{-3}$ suggest adequacy when using the rib biopsy technique.

Cohen (1973a) correlated the P concentration in pasture samples, blood, hair and bone. He found that there was a significant correlation ($r=.97$) between P in pasture and in dry, fat-free ribs. Correlation between P levels in pasture and in either hair or blood were not significant.

Rib P levels may not always accurately reflect skeletal P levels. As previously mentioned, Benzie et al. (1959) reported that sheep bones high in cancellous tissue, such as ribs, were the first to be depleted during P deficiency and to be repaired during P adequacy. Hoey et al. (1982) and Gartner et al. (1982) reporting on different aspects of the same study concluded that the use of cortical rib biopsy P concentration to differentiate between P deficient and P adequate animals is questionable. They found that cattle fed a diet containing .09% (DMB) ceased to grow after 19 wk although the lowest rib P levels in cortical rib biopsy samples varied from $169\text{--}181 \text{ mg P} \cdot \text{cm}^{-3}$ in P deficient animals and from $171\text{--}186 \text{ mg P} \cdot \text{cm}^{-3}$ in P adequate animals. Read et al. (1986b) concluded that P content of bone samples expressed as $\text{mg P} \cdot \text{cm}^{-3}$ (fresh basis) was a reliable sensitive indicator of the P status of

grazing crossbred cows sampled at different stages of their reproductive cycles.

Wilson and Hatfield (1974) developed a simple tail biopsy procedure and reported it to be a sensitive criterion of response to P source and level. Ash weight of caudal vertebrae responded linearly over a 112 d feeding period to different levels of P supplementation. These authors suggested that this technique in combination with other procedures could increase the accuracy of interpretation of data in regard to P nutrition.

(2) Mechanical properties of bone. Mechanical properties of bone, such as breaking strength, have been used by nutritionists in determining the bioavailability of minerals and establishing nutrient requirements for many years (Haugh et al., 1971; Crenshaw et al., 1981a). The response of bone to the variety of forces which it is exposed to throughout the life of an animal is a function of the mechanical properties of bone (Evans, 1973). Frankel and Burstein (1970) stated that the structure of bone responds to and is derived from its mechanical properties. Before discussing research involving P and the mechanical properties of bovine bone, a review of some of the basic principles of mechanics as they relate to the properties of bone may be helpful. A better understanding of principles involved in mechanical properties of bone would allow more accurate conclusions to be made concerning the effect of nutrients on mineralization and more accurate comparisons to be made among various experiments (Crenshaw et al., 1981a).

Stress and strain are created when a force acts upon a body. Strain is the change in length per unit length of a body when a force is applied; whereas stress is the force applied per unit area of the body. Bones are subjected continually to various forces acting upon them. The magnitude, direction, duration and rate at which a force is applied influences the responsiveness of the organ.

Behavior of materials, when subjected to mechanical loading, may be described in terms of four basic rheologic properties: 1) elasticity, 2) plasticity, 3) viscosity and 4) strength. In most materials, including biological materials like bone, these properties exist in combination.

Bone can be tested as either a structure or material. When tested as a structure, a force is applied until the bone fails. The breaking load (BL), which is a structural test, represents the force at which any further increase in force results in rupture. A material property of bone, stress, results from an applied load. The maximum stress the bone material can withstand is termed breaking strength (BS). Crenshaw et al. (1981a, b) and Lawrence (1986) have pointed out a major problem in the literature in that BL and BS, which are different, are mistakenly used interchangeably by numerous researchers.

Breaking load, the force required until failure of the bone, is dependent on both the size of the bone and quality of the material present. Breaking strength, however, takes into account not only the force required to cause failure, but the area and geometrical shape of area over which the force is applied (Crenshaw et al., 1981a).

Therefore, in order to calculate the BS, the area moment of inertia (I) of the bone must be determined. Breaking strength allows comparisons between anatomically similar bones which differ in size.

Equations for calculating I from simple measurements of an object have been derived for geometrical configuration of known shapes (circles, triangles, rectangles, etc.). However, bones are irregular in shape, making it difficult to determine the area and geometry for calculation of I. Some researchers have dealt with this by machining small uniform sections of known geometrical shape. When bone is mechanically tested in this manner, its material properties are examined. If the whole bone is tested, its structural (material and geometrical) properties are tested. Evans (1973) has stated that this type of testing is more representative of forces acting on bone in the live animal.

Lawrence (1986), using the SCADS computer program (Piotrowski and Kellman, 1973), determined the actual area and I of 2 cm thick equine third metacarpal (McIII) midshaft cross-sections irregardless of their geometry and found that elliptical estimates very closely approximated both the actual area and I of these sections when compared to circular estimates of these parameters. Values reported were 7.40, 3.55 and 3.66 cm⁴ for the circular, elliptical and SCADS I's, respectively. The use of the circular I overestimated the actual I by approximately 100%. Since I is used in calculating BS, erroneous values may be obtained if an appropriate estimate of I is not used.

Strength of materials can be determined by numerous types of tests. The most commonly used to evaluate the mechanical properties of bone by nutritionists is a flexure test (Baker and Haugh, 1979; Crenshaw et al., 1981a; Lawrence, 1986). In a flexure (bending) test, the bone is rested on two fulcra, one at each end of the bone, and a force is applied from above. A flexure test involves both compressive and tensile forces (Figure 2). Compressive forces tend to "push" an object together, while tensile forces tend to "pull" it apart. When a force is applied to bone in a flexure test, compressive forces are exerted on the top fibers while tensile forces are exerted on the bottom fibers. The neutral axis is the plane of material in the center which is neither in compression nor tension.

In a flexure test, force is plotted against deformation, which produces a force-deformation curve (Figure 3). Elastic deformation occurs in the initial linear phase of this curve. In this area, when the applied force is removed, the bone will return to its original shape, with no damage, as no permanent damage is done to the bone. However, the linear phase, the amount of force applied to the bone is sufficient to result in permanent damage to the bone. This is the region of plastic deformation (i.e., removal of the applied force will not result in return of the bone to its original shape). These rheological properties of bone give it its viscoelastic properties.

Traits that describe the mechanical properties of bone as determined by the flexure test include BS, BL, I, modulus of elasticity (E) and strain. Equations and variables used in the calculation of these parameters are presented in the material and

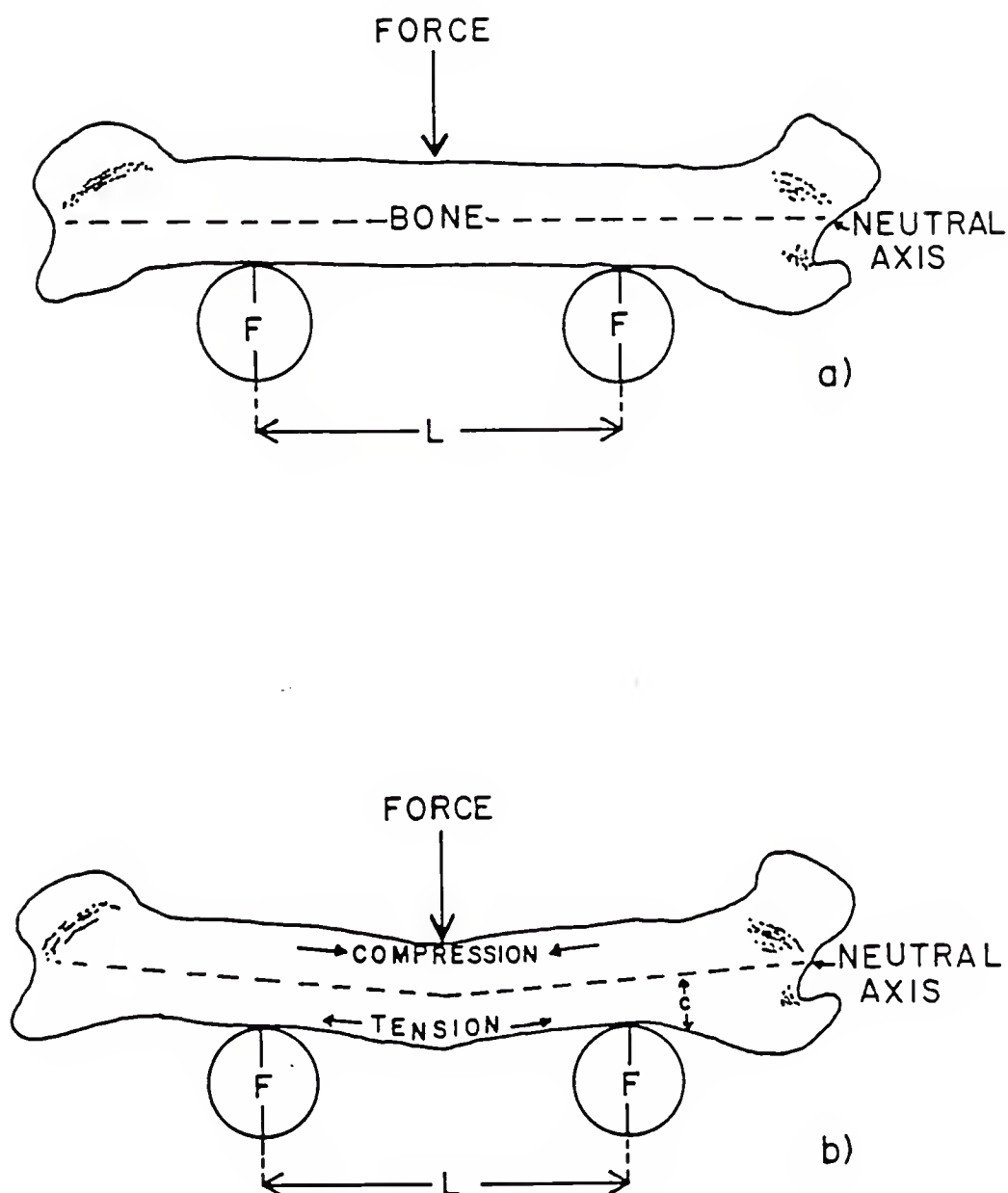


Figure 2. Diagram illustrating 3-point flexure test of bovine third metacarpal: a) pretest and b) during testing with force applied to the posterior surface. Neutral axis is represented by dashed line, c is distance from extreme outer fiber to neutral axis, L is the length between the roller supports, F .

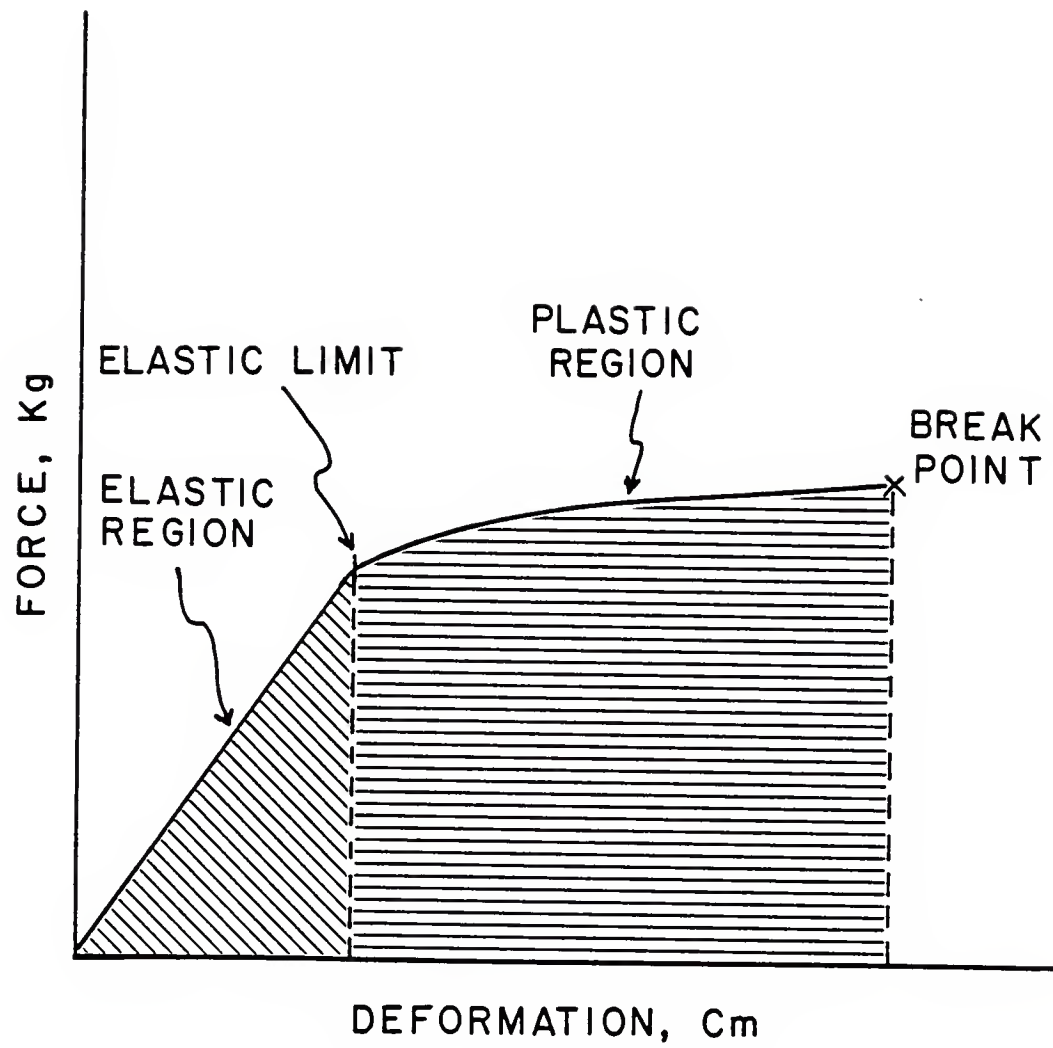


Figure 3. Force-deformation curve resulting from a flexure test.

methods section. Breaking strength, BL, I and strain have been previously discussed. Modulus of elasticity (E) (or Young's modulus) expresses stiffness under normal stress in the linear elastic region (Figure 3). It is the ratio of stress to strain. It is essential that deformation rates be included with results of flexure tests. As loading rate is increased, long bones have increased capacity to absorb energy (McElhaney, 1966), thus resulting in increased BS. Young's modulus is also dependent upon the rate of deformation and increases with increasing rates of deformation (Sedlin and Hirsch, 1966). Frost (1973) reported that bone may demonstrate nearly twice the stiffness at high speed loading rates compared to low rates. Very few animal nutritionists have reported loading rates in presentation of their results.

When assessing results of mechanical properties of bone derived from flexure tests, nutritionists must consider the nature of material being tested (i.e., nature of preparation, wet or dry; cancellous vs cortical bone). It was noted long ago that marked differences exist between bone in its wet and dry states. Dry bone has a slightly higher modulus of elasticity, is stiffer under elastic deformation and also has a higher ultimate stress--but it is quite brittle. In contrast, wet bone is quite ductile and allows considerable plastic behavior (bending past elastic limit) before fracture (i.e., dry bones are more nearly elastic and bend less upon testing than wet bones). As little as 10 min exposure in air and bone begins to show increased strength (Sedlin and Hirsch, 1966). Crenshaw et al. (1981a) have reported that nutritionists can use

either dry or wet bones (if treated uniformly) when examining the response of nutrient quality of the diet; however, wet bones would more closely represent bones as they exist in the animal.

Mechanical properties of bone also vary greatly with the age of the animal. Recent work with equine McIIIs (Bynum et al., 1971; El Shorafa et al., 1979; Glade et al., 1986; Lawrence, 1986) have shown that BS increases rapidly from day of birth until 5-7 yr of age, then declines. Lawrence (1986) also found dramatic increases in E of equine McIIIs in the first year of life, reaching a maximum value at 4 yr of age (from .98 to 3.6 GPa). From 4 to 27 yr the stiffness did not change significantly.

Several researchers have studied the relationship between stress and percentage of ash. El Shorafa et al. (1979) showed a positive correlation ($r=.58$) between BS and ash. Vose and Kubala (1959) reported that small increases in ash dramatically increased the BS in human femurs. These authors fit an exponential curve to their data as they felt a linear curve would not make "biological" sense (i.e., linearity would have predicted that a femur with a cortical ash of 60.8% would have a BS of zero). Currey (1969) later explained the lack of a linear relationship between BS and ash and the failure of bone to completely mineralize in that mineralization beyond an optimum value would decrease resilience of the bone and reduce its ability to resist dynamic loading. Currey (1969) suggested that the relation between bone mineral and collagen in bone matrix is responsible for the correlations of breaking strength and ash. Mineral is used to stiffen bone, but at low values, mineral crystals

disrupt the collagen molecules, when a load is applied. Currey (1969) also reported that, as ash values reach 63 to 68%, the organization of the mineral-collagen matrix produces the optimum strength. Currey (1969) also reported a linear relationship between E and ash content of bone. Schryver (1978) has also reported that differences in breaking strength in horses were positively related to specific gravity.

There are few reports in the literature that pertain to the mechanical properties of bovine bone in bending with respect to mineral nutrition (i.e., that follow the testing procedures previously described), whereas thorough investigations with equine (Schryver, 1978; Lawrence, 1986) and swine (Nimmo et al., 1980; Crenshaw et al., 1981b) have been reported.

Early studies by Becker and Neal (1930) reported data on bone strength of 15 mature cattle on low Ca and low P rations. Values in McIIIs ranged from 710 Kg in a Florida native cow receiving no supplemental Ca or P to 1581 Kg in a Guernsey cow receiving a bone-meal supplement. "Strength" values were also reported for third metatarsals, ribs, humeri and femora. These authors concluded that physical and chemical analyses of bone have a definite place beside mineral balance determinations in long term investigations of mineral requirements. Raulerson (1964) reported "breaking strengths" of right McIII in rams fed either .06 or .67% P (DM) of 71.9 vs 108.3 Kg, respectively. Method of testing was not reported. Deficient rams also had smaller, lighter bones than controls. More recently, Magomedov (1978) has reported that Black Pied heifers fed

either 20% above, 20% below or at recommended P intakes had McIII breaking strength values of 167, 175 and 171 MPa, respectively.

Mechanical properties of bone show differential responses to varying nutrient levels. Miller et al. (1962), working with baby pigs, indicated that bone stress reaches a maximum before BL in femurs. These workers fed increasing levels of Ca (.4, .8, 1.2 or 1.6%) and reported that BS increased with increasing Ca up to .8% Ca, while BL continued to increase up to 1.6% Ca. These data indicate that the .8% Ca level was adequate for optimum bone mineralization but more total mass of bone was deposited at higher Ca levels. Critical consideration must be given to these mechanical traits when assessing requirements for various classes of livestock.

(3) Noninvasive techniques. The Ca and P status at the skeleton of an animal can be simply determined by ash analysis of the bones. This method, however, necessitates either surgery or slaughter and thus may not be the preferred method of choice to the investigator.

Bone composition has been shown to be stable except when animals are subject to mineral deficiency or imbalance (Field et al., 1975), compared to flexibility of bone size and density. Therefore, any method from which all these parameters could be measured nondestructively would be valuable.

The accurate measurement of bone mineral content (BMC) is of interest to both clinicians and scientists. In vivo measurement of BMC is useful in evaluating sequential changes in bone density and other bone parameters. The quantification of bone mineral kinetics

is valuable in assessing skeletal changes that may be associated with normal skeletal development, gerontologic demineralization, restricted physical activity, nutritional influences, altered gravity exposure, radiation exposure, or endocrine and renal imbalancing conditions with secondary bone effects.

Three noninvasive techniques, radiographic photometry, photon absorptiometry and ultrasound, have received considerable attention and study in domestic animals over the last two decades for their ability to predict BMC and/or bone strength (Lawrence, 1986).

a) Radiographic photometry. Quantifying the mineral content of the skeleton has been puzzling researchers for centuries. Certain pathologic and histologic methods were used to measure skeletal mass; in vitro bone density measurements have been reported in the 19th century, but in vivo techniques have been developed in this century (Stoliker et al., 1976). The advent of radiology as a separate discipline, due to Roentgen's discovery of the x-ray in 1895, altered the approach to the problem.

Attempts to correlate actual bone mineral content with a radiograph were made as early as 1920 (Griffiths and Zimmerman, 1978). The technique for evaluation of BMC is based on the principle that the attenuation of x-radiation as it passes through bone and, therefore, the amount of light passing through a radiograph is related to the mineral content of the bone. Ardran (1951) and Lachman (1955), however, indicated that the use of conventional radiography in assessing BMC may require a 30 to 60% loss of bone mineral before a rarefaction could be visually detected. Excised

bone or stepwedges were later (1950s) placed on radiographs for comparative purposes. A reference standard must be exposed simultaneously with the bone in order to make direct photodensitometric comparison between x-rays. Several materials have been used as reference standards. These have included bone preparations, ivory, dipotassium hydrogen phosphate and aluminum (Al).

Omnell (1957) stated x-rays are absorbed by Al to the same degree as bone mineral; therefore, results can be expressed in mm of Al or BMC. Pridie (1967) reported that standards made of materials other than bone produce different absorption curves which may vary with the composition of the standard material and energy level of the x-rays. Regardless, Al stepwedges have been used successfully (Meakim et al., 1981; Landau et al., 1985; Lawrence, 1986).

Photodensitometric BMC determinations of defleshed chicken bones had coefficients of variation from .88 to 1.22%, similar to the values reported by Meakim et al. (1981). In vivo precision errors as low as 1% and as high as 15% have been reported (Lawrence, 1986). Cohn (1981) reported that predicting ash weight by radiographic photometry had a standard error of the estimate of $\pm 6\%$.

Soft tissue covering introduces much of the error in bone density estimations (Meema et al., 1964). To avoid errors associated with soft tissue, researchers have attempted to choose sites where soft tissue covering is minimal. Most radiographic determinations of BMC have been limited to the cortical area of the long bones, in which, as Meema et al. (1964), stated that cortical bone exists in a

virtually pure form (i.e., little or no cancellous bone). This makes the distal metacarpal a likely candidate for study in horses and cattle. Also, since cortical bone comprises 80% of the skeleton, information on cortical bone should reflect overall skeletal status (Meema and Meema, 1981).

Morphometric measurements of cortical bone in third metacarpals can also be obtained with radiography (Meakim et al., 1981; Lawrence, 1986). This allows serial assessment of changes in cortical area of these bones (as related to changes at the periosteal and endosteal surfaces).

Meakim et al. (1981) and Lawrence (1986) correlated radiographic bone aluminum equivalents (RBAE) as determined by radiographic photometry and BMC as determined by ashing of equine McIIIs from horses of varying breed and age. They found high correlations (from .88 to .95) using this technique.

Landau et al. (1985) studied the relationship between Ca and P retention in 2 wk old Fresian calves and metacarpal radiographic photometry. They found it to be a sensitive technique and concluded it was a reliable method for evaluating mineralization in the bovine.

Correlations of radiographic measurements and BMC have been good despite imperfection in bone geometry and variation in the composition of compact bone (Meema and Meema, 1981). Extton-Smith et al. (1969) reported a high correlation ($r=.85$) between radiographic cortical area and bone ash per unit length of 29 third proximal phalanxes of human subjects. Low correlations were found for both cortical thickness and cortical thickness/width ratio, which

is similar to the findings of Suttie et al. (1983) who reported poor correlations between cortical thickness/width ratio (or cortical bone index) of McIII of 69 red deer stags from 15 to 75 mo of age and mineral status, a technique developed by Barnett and Nordin (1960). However, it should be noted that these researchers correlated whole bone mineral instead of BMC at the point of radiographic measurement. Little (1984) reported that this cortical index method provided a sensitive criterion for assessing P status in cattle. Cattle fed either high P or low P (dietary levels not provided) were distinguished by a cortical bone index determined from radiographs, .68 vs .60 for high P vs low P, respectively, ($p < .05$). Medullary cavities tended to be greater in the low P group. Meakim et al. (1981) compared circular and elliptical cross-sectional indices determined from radiographs with BMC of equine McIIIs and reported correlations of .88 and .95, respectively. Lawrence (1986) reported a high correlation ($r = .89$) between BMC and radiographic circular index calculated using equine McIIIs of varying ages and breeds.

b) Photon absorption. A specialized technique, photon absorption, for determining bone mineral mass utilizing the absorption and recording of radiant gamma energy was first reported by Cameron and Sorenson in 1963. The technique is based on the same principle as that of the roentgenographic method, namely that the mass of bone mineral present is directly proportional to the amount of photon energy absorbed by the bone. As the bone is scanned, the number of photons reaching the scintillation detector is plotted in a transmittance curve. The area of the curve is proportional to BMC

(Smith et al., 1972). Calibration of photon absorption units is accomplished by reference standards that simulate bone and serve as a reference guide for BMC calculations (Mazess, 1981).

Cameron and Sorenson (1963) indicated that this method was distinguished from radiographic photometry procedures in that 1) the transmission of the photon beam is measured directly by counting techniques, by means of a scintillation detector system; 2) the photon beam is monochromatic and highly collimated; and 3) the effect of the tissues around the bone are accounted for (i.e., fat, muscle, cartilage and other soft tissues appear similar to each other and to the absorption pattern of water). These authors point out that these factors abolish errors associated with radiographic methods, including variation in x-ray films and subsequent development techniques, soft tissue effects, uncertain absorption coefficients and scattered radiation.

Either single or dual photon absorption units are available presently. In single photon absorption, the radiation source (usually ^{125}I) is rigidly coupled to a NaI crystal scintillation detector system. The detector and source simultaneously scan the bone transverse to its longitudinal axis. Soft tissue effects are accounted for by use of a tissue equivalence bag which has a similar absorption pattern as soft tissue. Dual photon absorption utilizes the emission of two separate photon energies which discriminate between bone mineral and soft tissue, thus dual photon absorptiometry provides the advantage of not having to surround the bone with a

tissue equivalent. Radionuclide sources used in dual photon absorptiometry include $^{125}\text{I}/^{241}\text{Am}$.

Repeatability and accuracy of bone mineral mass estimations using photon absorptiometry have been actively studied in many species. Using parafin blocks containing homogeneously mixed CaCO_3 in known amounts, Cameron and Sorenson (1963) reported accuracy of mineral content estimations to $\pm 3\%$. These same authors reported a reproducibility of within 3% when 137 human female subjects were scanned. Smith et al. (1972) reported coefficients of variation of 2.5 and 2.7% for cortical and trabecular bone, respectively, on 21 repetitive BMC measurements of a normal human radius over a 2 wk period. Coefficients of variation associated with direct photon absorption from 2.2 to 6.6% for 10 BMC/width measures on six rats have been reported (Sanchez et al., 1981). These authors also reported correlations between BMC/width and whole femur ash weight of $r=.93$ and dry weight of $r=.96$. Excellent correlations between bone mass as determined by photon absorption and bone ash ($\text{g}\cdot\text{cm}^{-1}$) as determined by ashing have been reported in poultry ($r=.93$) (Meyer et al., 1968) and in canine and equine species ($r=.99$) (Wentworth et al., 1971).

Photon absorption has been used as a tool to observe the effects of dietary concentrations of Ca on BMC and to observe remineralization of bone following the change from a Ca deficient to a Ca repletion diet (Krook et al., 1971; Wentworth et al., 1971). Henrikson et al. (1970) used photon absorption to study the effect of dietary fluoride on bone of dogs. Jeffcott (1985) recently used

photon absorptiometry to assess bone quality (BMC) as influenced by chronological age, partial weightlessness and forced walking and trotting exercise in horses. Also, Holmberg et al. (1985) have conducted long term studies using photon absorption in dairy cattle to monitor bone mineral changes in coccygeal vertebrae during different lactations and aid in studying parturient paresis.

In vivo bone mineral analysis by photon absorptiometry in animals has been found to be a sensitive, reproducible, noninvasive and available method, and with the advent of large animal scanners as described by Jeffcott (1985) will become a powerful tool for serially monitoring changes in bone mass of large animals.

c) Ultrasound. Sound waves travel through bone with a velocity ($\text{m}\cdot\text{s}^{-1}$) that reflects the material and structural properties of bone (Gerlanc et al., 1975). Pratt (1982) has termed this velocity the "effective" velocity; whereas, Jeffcott and McCartney (1985) have used the term "apparent" velocity, as the actual path the sound waves take through the bone has not been revealed.

The "effective" or "apparent" velocity is determined by placing transducers that transmit and receive sound waves from an ultrasonic wave generator on opposing surfaces of the bone. The time it takes for the propagation of a sound wave to travel from the transmitting transducer through the bone to the receiving transducer (t) is divided by the width of the bone being tested (d). This calculation yields an estimate of velocity (v) of sound through the bone. Lawrence et al. (1986), to better approximate the flight of the ultrasonic beam through bone, compared the "effective" velocity with

that of velocity based on area indices at the point of transmission. They reported the more accurate the estimate of area the higher the correlation between ultrasound and BMC.

Using fresh rectangles of bovine cortical bone, Rich et al. (1966) studied the relationship between ultrasound and chemical properties of bone. They reported a correlation between $\text{mg Ca} \cdot \text{cm}^{-2}$ in bovine cortical bone and ultrasound velocity of $r=.99$. Bokman et al. (1983) measured ultrasonic velocities at the midpoints of McIII of eight Angus cows and eight Hereford heifers. They reported a correlation of .63 between bone density and ultrasonic velocity. Recently, Lawrence and Ott (1985) reported a correlation of .92 for BMC and ultrasound in 25 pairs of McIII from equine cadavers of varying breeds and ages. Jones (1984) has suggested that ultrasound is a better indicator of BS than BMC.

Abendschein and Hyatt (1970) studied the relationship between E in standardized specimens of human cortical bone and ultrasound. The modulus of elasticity of bone has been studied in its relationship to the functional capacity of bone and directly related to its breaking strength. Abendschein and Hyatt (1972) proposed that ultrasonic velocity is a function of E and the density of bone. These authors found a linear relationship between E determined by testing guinea pig femurs loaded in compression and the ultrasound velocity. Abendschein and Hyatt (1972) further state that ultrasonic velocity can accurately quantitate E and other physical properties in bone.

Glade et al. (1986) reported BS calculated from sound velocity measurements in equine McIIIs were within 2% of BSs measured

mechanically ($r=.91$). Lawrence and Ott (1985) reported a correlation of .82 between sound velocities through soft tissue-free equine cadaver McIIIs and their measured BS. Jeffcott and McCartney (1985) compared ultrasonic velocities with specific gravity of McIIIs obtained from 18 normal equine cadavers. They reported an apparent linear correlation but noted that much of the variation in velocity was due to age.

Ultrasound appears to be both accurate and reproducible. Rabin et al. (1983) reported on ultrasound data collected on 262 horses. They reported their accuracy to be better than 1% and reproducibility to be better than 0.5%. Comparable results have been reported (Greenfield et al., 1981).

None of the techniques previously discussed (i.e., radiographic photometry and photon absorption) that estimate bone mineral density consider the architectural framework in which the mineral fraction resides. Breaking strength (and other physical properties of bone) is a function of mineral lattice and collagen orientation and organization (Carter and Spengler, 1978), which are not detected by x-rays or gamma-rays, but which affect sound transmission in bone. Therefore, assessment of structural integrity, quality, change and development in long bones may be best monitored by ultrasound instead of techniques which measure mineral parameters of bone only (Glade et al., 1986).

Feces

Fecal samples are easily obtained, and, since P homeostasis in ruminants is achieved to a great extent in the gastro-intestinal

tract by controlling the secretion and reabsorption of salivary P, fecal losses should be related to intake or absorption (Clark et al., 1973). Several reports in the literature suggest that the status of P nutrition in ruminants may be assessed by fecal collection methods (Moir, 1960; 1966; Belonje, 1978). It has been suggested that fecal grab samples from both cattle and sheep may be useful in estimating the P content of the pasture being grazed.

Problems with the use of a fecal grab sample may be encountered if, for example, large differences in feed intake are seen. Total fecal output may differ, resulting in a difference in total mass of P excreted ($\text{g} \cdot \text{d}^{-1}$), but with no apparent difference in % P in the samples. Read et al. (1986a) indicated that fecal P concentration determined from fecal grab samples was not sensitive as a P status indicator due to differences previously mentioned.

Cohen (1974) reported that Angus steers fed carpet grass, with or without alfalfa and supplemented with various amounts of sodium orthophosphate, had P intakes related to total daily fecal P excretion ($r=.98$). Cohen (1974) suggests that the P intake of cattle may be estimated from regression equations of the intake of a specific feed source on total daily P excretion, using different equations for different feed sources in order to eliminate differences in digestibility. Cohen (1974) also stated that selective grazing by sheep and the existence of plant species in a natural or mixed pasture with varying digestibilities, which may vary with season or time of day, make fecal collection very unreliable for assessing the P status of grazing animals.

Moir (1966) reported seasonal fluctuations in P and crude protein (CP) in feces from cattle in four areas of Queensland, while using these determinations as indicators of P and protein intakes. Fecal CP levels did not exhibit the marked drop during the dry season shown from two of the other areas, and a markedly increased ratio of fecal protein to fecal P occurred over this period. The fecal P was reduced to about half the wet season level. Moir (1966) predicted pasture contents of P and Ca from fecal samples. Lebdoesoekojo (1977) using fecal grab samples found fecal P to be highly correlated with pasture P ($r=.84$) in beef cattle grazing mainly *Melinis minutiflora* at Carimagua, Colombia.

Saliva

Salivary P concentration of P deficient animals was reported to be approximately 50% that of animals supplemented with P (Clark, 1953). However, it has been suggested that salivary P concentration is not a reliable indicator of P status due to variation in P concentration caused by varying amounts of saliva secreted daily by cattle (Anon., 1976, cited in Loxton et al., 1983). This conclusion is consistent with the work of Perge et al. (1983) who found wide variation in salivary P concentration as affected by dietary supply of Ca and P as well as time of sampling.

CHAPTER THREE MATERIALS AND METHODS

General Protocol

The objectives of this experiment were to determine the effect of different phosphorus (P) concentrations on performance of growing beef heifers and evaluate criteria for assessing P status in these animals. The experiment consisted of a P depletion phase (270 d), an adaptation period to the basal P supplementation diet (10 d) and a P supplementation phase (ranging from 245 to 492 d in length; common experimental endpoint was day 245 of supplementation phase for nonpregnant and 3 wk postpartum for pregnant heifers). For the entire duration of the experiment (525 to 772 d), all animals were housed at the Animal Nutrition Laboratory, University of Florida, Gainesville Experiment Station.

Depletion Phase

Fifteen weaned Angus heifers,¹ 7 to 8 mo of age, 160±3 kg initially were randomly assigned to three pens (5 animals·pen⁻¹) and housed under dry lot conditions on concrete floors in a covered barn. All heifers were allowed ad libitum intake of the P depletion diet² (Table 2) and free access to water throughout the 270 d depletion phase (October 16, 1982, to July 12, 1983). The P

¹ Obtained from University of Florida AREC, Quincy, FL.

² Prepared at Seminole Stores Incorporated, Ocala, FL.

Table 2. Composition of basal diets fed during phosphorus depletion and supplementation phases.

Ingredient	Amount ^a	
	Depletion Phase	Supplementation Phase ^b
Citrus pulp, %	30.0	35.0
Cottonseed hulls, %	30.0	17.5
Soybean hulls, %	-	20.0
Coastal Bermuda Hay, ground, pelleted, %	15.5	-
Cardboard paper, ground, %	10.5	11.0
Cane molasses, %	10.0	10.0
Animal fat, %	-	2.5
Urea, %	2.0	2.0
Mineral premix, ^c %	2.0	2.0
Vitamin A and D, ^d %	+	+
Total	100.0	100.0
Chemical composition ^e		
Calcium, %	.60	.62
Phosphorus, %	.10	.12 ^f
Magnesium, %	.32	.34
Potassium, %	1.17	1.31
Sodium, %	.28	.24
Zinc, mg/kg	37.60	39.40
Copper, mg/kg	13.80	14.90
Cobalt, mg/kg	.13	.15
Molybdenum, mg/kg	4.30	4.70
Crude protein, %	10.83	11.91

^a Dry-matter basis (DMB).

^b MonofosTM (International Mineral and Chemical Corp., Mundelein, IL) added to the basal diet at expense of cane molasses to achieve .20% total P in supplemented diet.

Table 2--continued.

- ^c Supplied mg per kg diet DM (compound, element): Sodium selenite (Na_2SeO_3) .21, .10; Nickel sulfate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) 8.96, 2; Stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) 3.80, 2; Chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) .51, .10; Ammonium vanadate (NH_4VO_3) .23, .10; Potassium iodate (KIO_3) .17, .10; Sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) 12.5, 5; Cobalt carbonate (CoCO_3) .22, .10; Cupric sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 24, 4; Manganese oxide (MnO_2) 15.8, 10; Zinc oxide (ZnO) 37.5, 30; Ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) 49.7, 10; Sodium Chloride (NaCl) 2,700, 2,700; Calcium carbonate (CaCO_3) 450, 180; Potassium Chloride (KCl) 6,350, 3,300; Dyna-Mate (18%K, 22%S, 11%Mg; International Mineral and Chemical Corp., Mundelein, IL) 11,200 (2,000, 2,425, 1,223).
- ^d Supplied per kg of diet: 2200 USP units Vitamin A palmitate and 440 USP units Vitamin D_3 .
- ^e By laboratory analysis.
- ^f Average phosphorus (P) content of basal diet; P content varied from .11 to .13% during course of supplementation phase, dry matter basis.

depletion diet was formulated to be low in P yet provide adequate energy, protein, and other minerals and vitamins to promote $.5 \text{ kg} \cdot \text{d}^{-1}$ gain. The mineral mix and vitamin premix used in the depletion diet (as well as in the supplementation phase) were prepared at the Animal Nutrition Laboratory. Calcium to P ratio of P depletion diet was 6:1. All animals were dewormed at initiation and midpoint of the depletion phase. Animals were bled periodically by jugular vein puncture using 15 gauge California Bleeding needles¹ (Table 3) according to methods described by Fick et al. (1979).

Supplementation Phase

Prior to the beginning of the P supplementation phase all heifers were allowed a 10 d adaptation period to the basal diet.² On July 22, 1983, 14 heifers (16 to 17 mo of age; $210 \pm 6 \text{ kg}$, initially) were weighed, dewormed and randomly allotted ($7 \text{ animals} \cdot \text{group}^{-1}$) to one of two dietary levels of P: 1) continuation of low P (LP) basal diet containing .12% P (dry matter basis, DMB) or 2) high P (HP) diet which consisted of the basal diet supplemented to provide .20% total P in the diet dry matter (Table 2). The basal diet was formulated to be low in P yet provide adequate energy, nitrogen, other minerals and vitamins to promote $.5 \text{ kg} \cdot \text{d}^{-1}$ gain. Calcium to P ratios ranged from 3.1:1 in the HP diet to 5.2:1 in the LP diet.

Animals were allowed ad libitum intake of their respective diets for the initial 210 d of the P supplementation phase (July 22, 1983,

¹ Jensen-Salsberg Laboratories, Inc., California Bleeding Needles, Kansas City, MO.

² Prepared at High Springs Milling, High Springs, FL.

Table 3. Bleeding dates and blood constituents sampled during depletion phase.

Date	Constituent ^a
December 20, 1982 (66) ^b	S
February 16, 1983 (124) ^c	S,P
April 13, 1983 (180)	S,P,W
May 25, 1983 (222)	S,P,W
July 6, 1983 (264)	S,P

^a S = serum, P = plasma, W = whole blood.

^b Day of P depletion phase.

^c Heifer 232 died February 14, 1983, of causes not related to experimental diets.

to February 16, 1984). Throughout this period, feed intake was recorded daily for each group. Feed offered was reduced to 6.8 kg·head⁻¹·day⁻¹ (as-fed) until May 10, 1984, at which time it was further reduced to 4.5 kg·head⁻¹·day⁻¹ (as-fed). Feed intake was restricted in an attempt to limit body condition in order to minimize potential calving difficulties. Feed offered was periodically sampled for chemical analyses. Heifers were provided free-access to water throughout the supplementation phase. Heifers were weighed at 2 wk intervals at 0800 h prior to feeding.

Ovarian activity of heifers was determined monthly by rectal palpation from October 14, 1983, to March 16, 1984 (6 palpations). Heifers were observed each morning (0730 h) for at least 30 min for signs of sexual activity in both P depletion and supplementation phases. Heifers were exposed twice daily (0730 and 1700 h) for a 30-min period to a bull from October 28 to November 23, 1983. From November 24, 1983, until removal of the bull (February 26, 1984), all heifers were placed with the bull overnight (i.e., from 1700 to 0730 h) in a small dirt dry lot which had been cleared of all vegetation.¹ The bull had access to the heifers for a total of 120 d. After the final palpation (March 16, 1984) two heifers from both LP and HP groups were diagnosed as nonpregnant² and were slaughtered on March 22, 1984, and samples recovered (Table 4).

¹ Monsanto Corporation, RoundUp[™], St. Louis, MO.

² Upon examination of the reproductive tracts of these heifers obtained at slaughter, heifer No. 172 of the LP group was found to be pregnant with a 55 to 60 d conceptus. All other tracts were found to possess ovaries with follicles or CL present except heifer No. 278 of the HP group. This heifer was diagnosed as having a prepubertal uterus at this time.

Table 4. Samples obtained at slaughter of experimental animals.

-
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1. Third metacarpus (right and left)
 2. Tail (coccygeal vertebrae V, VI and VII)
 3. 12th rib (right and left)
 4. Liver (right lobule)
 5. Kidney
 6. Heart
 7. Longissimus dorsi muscle
 8. Rumen contents with fluid component^a
 9. Abomasal contents with fluid component^a
 10. Reproductive tracts^b
-

^a Not collected from animals slaughtered on 3-22-84.

^b Only collected from animals slaughtered on 3-22-84. Tracts were visually examined for any abnormalities that may have been associated with the nonpregnant status of the heifers.

The remaining pregnant heifers (5 per group; 10 total) were maintained until 3 wk postpartum at which time they were slaughtered and samples obtained (Table 4). Calving occurred between August 11, 1984 (birth of first calf) and November 3, 1984 (birth of last calf). Calves were weighed at day of birth (DOB) and 3 wk of age. Blood samples were obtained from both heifers and calves at birth and 3 wk postpartum. Colostrum samples were taken from heifers within the first 6 h after birth of calves. Milk samples were collected 3 wk postpartum. Heifers that lost calves due to death were milked out by hand twice daily (0800 and 1700 h) until 3 wk postpartum.

Heifers were periodically sampled throughout the P supplementation phase (Table 5). Animal stress and excitement were avoided or minimized during and prior to tissue sample collection. Sample collection was performed during the early morning (from 0730 to 0800 h).

Blood samples were obtained by jugular vein puncture as described in the depletion phase. Blood plasma was obtained using lithium citrate (20% w/v; .1 ml·10 ml blood⁻¹) in the collection tubes. Blood serum was obtained using serum separation tubes.¹ Whole blood was collected in plastic tubes prepared as for plasma and frozen (-15 C) for subsequent analysis. Plasma and serum were separated by centrifugation (2500 rpm for 20 min) 20 min after collection and frozen (-15 C) until further analysis.

¹ Becton-Dickinson Co., Vacutainer Serum Separation Tubes (SST), Rutherford, NJ.

Table 5. Sample collection during P supplementation phase.

Date ^a	Samples Collected ^b
October 8, 1983 (79) ^c	P, S, W
November 18, 1983 (120)	P, S, W, BB, LB, H, F
January 22, 1984 (186)	P, S, W
March 15, 1984 (238)	P, S, W, H, F, Sv
May 10, 1984 ^d (294)	P, S, W, BB, H, F, Sv, RF

^a Does not include serum, plasma, whole blood and colostrum/milk samples collected at parturition and 3 wk postpartum from heifers and calves.

^b P = plasma, S = serum, W = whole blood, BB = rib bone biopsy, LB = liver biopsy, H = hair, F = fecal grab sample, Sv = Saliva, RF = rumen fluid.

^c Day of P supplementation phase.

^d This collection included only 5 heifers·group⁻¹ (10 total) as four heifers were slaughtered on 3-22-83.

Rib cortical bone biopsy samples were obtained using a modified version of the surgical procedure described by Little (1972b). Samples were obtained from the 12th rib of all animals (left side on d 120; right side on d 294 of the P supplementation phase) using a 1.4 cm diameter trephine. Trephine depth was set to allow both medial and lateral aspects of the intercostal appendage to be collected. These samples were wrapped in .9% saline soaked gauze and frozen (-15 C) until further analysis.

Liver samples were taken in vivo using the liver biopsy technique described by Fick et al. (1979). These samples were frozen (-15 C) immediately after collection.

Hair samples were obtained by clipping at 30 cm² area on the left flank of the animal using electric clippers. Hair was stored in plastic bags until further analysis.

Fecal grab samples were obtained by using plastic rectal palpation gloves while animals were restrained in a squeeze chute. Approximately 100 g of feces were collected and placed in plastic bags and frozen (-15 C) until subsequent analysis.

Salivary samples were obtained using a modification of a technique described by Murphy and Connell (1970). Approximately 8 to 10 ml of saliva per heifer was collected using a suction device. Saliva was transferred into plastic tubes and centrifuged (3000 rpm for 15 min) to remove food particles and soil, then frozen (-15 C) until further analysis.

Rumen fluid (in vivo) was obtained while animals were restrained in a squeeze chute using a stainless steel Frick speculum (3.02 cm

inner diameter, 51 cm in length) which was placed in the mouth through which a stomach tube (1.9 cm inner diameter, 3 m in length) was passed into the rumen. Approximately 25 ml of rumen fluid was collected. This was frozen (-15 C) in plastic bottles until further analysis.

Colostrum and milk samples were collected as previously described. Prior to collection, the udders of all cows were cleaned to minimize possible mineral contamination. Approximately 100 ml was collected and frozen (-15 C) in plastic bottles until further analysis.

Samples collected at slaughter of all animals are shown in Table 4. All soft tissue (including periosteum) was removed from the right and left third metacarpals (McIII) following slaughter. When removing surrounding tissues including fibrous periosteum, great care was taken so that the mineral fraction would not be disrupted. While removing surrounding soft tissues, McIII were always kept submerged in .9% saline. When complete, McIII were wrapped in .9% saline soaked gauze and frozen (-15 C) to maintain original moisture content until further analysis. Metacarpals were prepared in this manner to ensure that biomechanical tests would be performed on "wet" bones which resemble more closely the bones as they exist in the animal (Sedlin and Hirsch, 1966). Right and left 12th ribs from the transverse lumbar process to the distal articulating cartilaginous ends were removed and prepared in the same manner as McIII. Tails (with hide removed) were also wrapped in .9% saline soaked gauze at slaughter and frozen (-15 C) until further analysis.

Approximately 100 g of liver (right lobule), kidney (capsule intact), heart and longissimus muscle were collected at slaughter following procedures outlined by Fick et al. (1979).

Rumen and abomasal samples were collected by making a small incision through the respective compartments and extruding material from within into 150 ml plastic bottles and frozen (-15 C) until subsequent analysis.

Noninvasive and Mechanical Analysis of Bone

Photon Absorption

A Lunar Radiation Corporation¹ analyzer was employed for dual photon absorptiometry bone analysis which utilizes a highly collimated radionuclide source emitting radiation at two separate energies. As McIII were scanned, radiation was transmitted, scattered or absorbed depending upon the amount of mineral present. Dual photon absorptiometry is designed to account for soft tissue effects by using gamma rays at two different energies. Metacarpals were submerged in .9% saline which has an absorption pattern similar to soft tissue. Whole McIII were scanned yielding information on such parameters of interest as estimated bone mineral content (g) and bone mineral density ($\text{g}\cdot\text{cm}^{-2}$). These parameters can be estimated for the entire bone or specific sections as the scan line is 4.5 mm in width. Estimated bone mineral content and bone mineral density were

¹ Lunar Radiation Corporation, Bone Mineral Analyzer Dual Photon Rectilinear Scanner Model DP3, Madison, WI.

determined for whole McIII and two separate diaphysis sections. Five scan lines were read (2.25 cm) at both the midpoint and 3 cm proximal to the midpoint (approximately 1.125 cm on either side of these points).

Radiographic Photometry

Anteroposterior (AP) radiographs were taken of all McIII with an aluminum stepwedge¹ exposed simultaneously as a reference standard. Radiographs were obtained using mobile x-ray equipment² and veterinary x-ray film³ in cassettes with compatible intensifying screens. Voltage, amperage, exposure time, film focal distance, film developing and processing were performed as described by Meakim et al. (1981). To ensure uniformity, all McIII radiographs were shot on a single day. Photometric scans of the stepwedge and bone (at the midpoint and 3 cm proximal to the midpoint) were performed using a densitometer⁴ coupled to a microcomputer⁵ and digitizer.⁶ Peak lateral, medial and midpoint radiographic bone aluminum equivalents (RBAE) were averaged and this value reported as RBAE. This provided an estimate of bone mineral content.

¹ Atomic Products Corp., Atomic Lab Div. Center, Al Stepwedge Penetrometer, Moriches, NY.

² Universal X-Ray Products, Easymatic Super 325, Chicago, IL.

³ 3M Corp., Animal Care Products, Standard Veterinary X-Ray Film, St. Paul, MN.

⁴ Southern Micro Instruments Inc., Microcomp Digital Image Analysis System, Atlanta, GA.

⁵ Zenith Data System Corporation, Model Z-150 PC, St. Joseph, MI.

⁶ Houston Instrument, Hipad Digitizer, Austin, TX.

Ultrasound

Sound travels through bone with a velocity that reflects the material and structural properties of bone (Gerlanc et al., 1975). High frequency ultrasound was used to evaluate BMC and bone strength by measuring time elapsed and distance over which a standardized pulse of sound transmitted mediolaterally through McIII traveled (Figure 4). Ultrasound measurements were made at both the midpoint and 3 cm proximal to the midpoint, identical to those of photon absorption and radiographic photometry. An ultrasonic pulse generator¹ transmitted and received signals via matched KB Aerotech 1 MHz transducers mounted on an electronic digital caliper.² For all measurements a liberal quantity of coupling medium was used to ensure good sound transmission. It was only possible to obtain consistent transmission of sound over the region of the McIII shaft that had reasonably parallel sides. The elapsed time between transmission and reception of a signal (t , μs) was displayed on an oscilloscope.³ The mediolateral diameter of McIII (d , mm) was measured simultaneously by the electronic caliper. The "effective" ultrasonic velocity (U , $\text{m}\cdot\text{s}^{-1}$) of sound pulses through the McIII was calculated using the expression $U = d/t$.

Mechanical Testing

After all noninvasive tests were complete mechanical properties of all bones (McIII and ribs) were determined by three-point bending

¹ Panametrics, Pulser Receiver 5055 PR, Waltham, MA.

² Fowler, Ultracal Electronic Calipers, Detroit, MI.

³ Ballantine Lab Incorporated, Model 1023AB, Boonton, NJ.

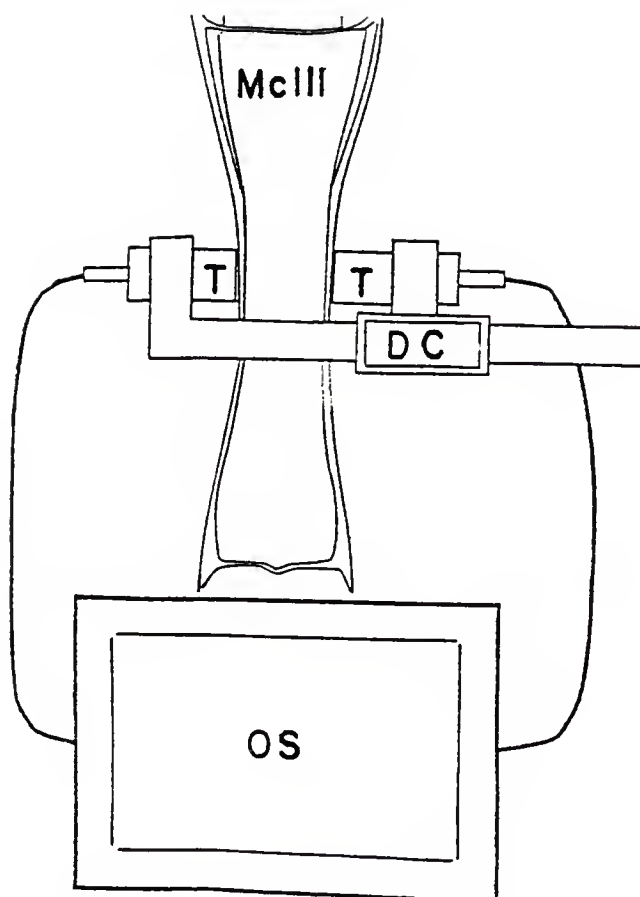


Figure 4. Diagram illustrating the use of ultrasound on bovine third metacarpals. McIII = third metacarpal, T = transducer, DC = digital caliper and OS = oscilloscope. Adapted from Jeffcott and McCartney (1985).

using an Instron Floor Model Testing Machine.¹ The "wet" bones were treated as a beam resting on two supports 13 and 10 cm apart for McIII and rib, respectively (Figure 2). Force was applied (posterior surface of McIII; medial surface of rib) at a constant deformation rate ($50 \text{ mm} \cdot \text{min}^{-1}$) to all bones and a strip chart recorder was used to plot the force-deformation curve (Figure 3), where chart movement was proportional to crosshead movement.

Broken McIII were placed in 70% ethanol solution (w/v) for 72 h to aid in removing bone marrow, then reassembled using Elmer's² glue. Two 2 cm cross-sections were then removed, one at the point of loading (midpoint of McIII diaphysis) and one 1 cm proximal to this section (3 cm proximal to the midpoint) using a table band saw. Preparation of the 2 cm McIII diaphysis cross-sections involved placing McIII in containers slightly larger than the McIII. Liquid urethane,³ which solidifies, was poured around McIII to provide a uniform, flat surface so that the two 2 cm cross-sections could be accurately cut from McIII. Caliper cross-sectional measurements from the 2 cm sections were anteroposterior (AP) bone diameter (B), AP medullary diameter (b), lateromedial (LM) bone diameter (D) and LM medullary diameter (d) (Figure 5). Diameters of cross-sections were

¹ Instron Corporation, Model 1125, Canton, MA.

² Borden Incorporated, Elmer's Glue-All, Columbus, OH.

³ Bradson Auto Marine Supply, Incorporated, Brad Foam, Pinellas Park, FL.

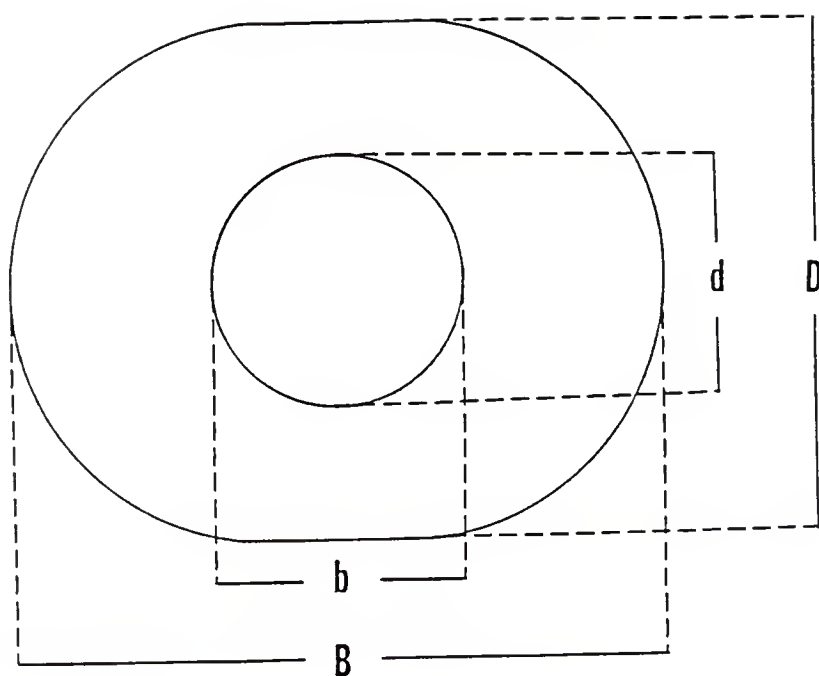


Figure 5. Cross-section of bovine third metacarpal (McIII) illustrating the anteroposterior (AP) outer (B) and inner (b) diameters and the lateromedial (LM) outer (D) and inner (d) diameters used in calculating moment of inertia of an ellipse (MIE), circular area index (CAI) and elliptical area index (EAI). Adapted from Lawrence (1986).

used in an equation ($\pi/64 (BD^3 - bd^3)$) for calculating the area moment of inertia of an ellipse (MIE, cm^4). Equations for calculating breaking strength (BS, MPa) and Young's modulus (E, GPa) were $BS = (F)(L)(C)/4(MIE)$ and $E = (F)(L^3)/48(MIE)(D)$, where F = force (kg), L = distance between supports the bone rests on (cm), C = distance from neutral axis to extreme outer fiber (cm) and D = deformation (cm). Force and deformation values were determined directly from force-deformation curves (Figure 2).

The McIII diaphysis cross-sections were photographed with a reference standard and the actual area (PAI, mm^2) of the sections irregardless of geometry determined using a planimeter.¹ Equations for calculating cross-section area indices (mm^2) for known geometrical shapes were ($B^2 - b^2$) for circular area index (CAI) from bone and radiographic area index (XRI) from AP radiographs and (BD-bd) for elliptical area index (EAI) from bone. The 2 cm cross-sections were dried for 12 h at 105 C then ether extracted in a soxhlet until no lipid material was observed in the ether (minimum 48 h). Sections were then ashed at 600 C for 12 h. Bone ash was expressed as g per 2 cm section and defined as BMC.

Chemical Analyses

Feed, Feces, Rumen Fluid and Rumen/Abomasal Contents

Feed samples were processed according to methods described by Fick et al. (1979) and analyzed for mineral content (Table 6). Crude

¹ Numonics Corporation, Model 1250-1, Lansdale, PA.

Table 6. Mineral analyses performed on samples.

Sample	Element(s)
Bone	Ca, P, Mg
Colostrum, milk	Ca, P, Mg, K, Na, Zn, Cu
Feces, rumen/ abomasal contents	Ca, P, Mg, K, Na, Zn, Cu, Co, Mo
Feed	Ca, P, Mg, K, Na, Zn, Cu, Co, Mo
Heart, kidney, liver, muscle	P, Zn, Cu, Fe, Mn, Co, Mo
Hair	P, Zn
Plasma, serum, whole blood	Ca, P, Mg, K, Na, Zn, Cu
Rumen fluid	P
Saliva	P

protein concentrations in feed were determined by measuring total nitrogen, following the method of Technicon Industrial System (1978). Feces as well as ruminal and abomasal contents (with fluid component) were placed in aluminum pie plates and dried in a forced-air oven at 60 C for 48 h. These samples were then processed and analyzed for mineral content (Table 6) in the manner previously described for feed samples.

Two milliliter aliquots of vortexed rumen fluid were digested on hot plates in narrow mouth 50 ml Erlenmeyer flasks using 7 ml HNO_3 and 4 ml of HClO_4 (70%) until the solution was clear and dense HClO_4 fumes were evolved. Samples were then refluxed for 30 min. Samples were filtered, brought to constant volume and diluted for subsequent mineral analysis (Table 6).

Animal Tissues

Blood plasma and serum were deproteinated with 10% trichloroacetic acid (TCA) and 1% Lanthanum chloride (LaCl_3) and analyzed for mineral content (Table 6). Whole blood was prepared for mineral analysis by digesting 1 ml aliquots of whole blood in 7 ml HNO_3 and 4 ml HClO_4 (70%) in the same procedure previously described for rumen fluid and mineral content determined (Table 6). Hemoglobin was determined colorimetrically¹ and hematocrit (PCV) by a micro-hematocrit method.

¹ Becton-Dickinson Co., Unopette 5857, Rutherford, NJ.

Two milliliter aliquots of vortexed saliva were digested in 4 ml HNO_3 and 3 ml HClO_4 (70%) using the same procedure previously described for whole blood and rumen fluid.

Liver (and liver biopsy), kidney, heart and longissimus muscle were prepared according to methods described by Fick et al. (1979) and analyzed for mineral content (Table 6).

Hair was prepared for mineral analysis (Table 6) using the method described by Kiatoko et al. (1982).

Colostrum and milk samples were processed using a modified version of the method Fick et al. (1979) described for forage. Approximately 10 g of colostrum or milk (which had been well mixed) was placed into weighed crucibles and heated to dryness on a hotplate. This was then placed in a 105 C oven for 8 h, cooled and a dry weight obtained. Samples were then ashed in a muffle furnace at 600 C for 12 h and mineral content subsequently determined (Table 6).

Coccygeal vertebrae V, VI and VII were located in tail samples and removed with a stainless steel scalpel. All soft tissue was removed. Bone density ($\text{g}\cdot\text{cm}^{-3}$) was determined for all vertebrae and rib bone biopsies using a Mettler density determination kit.¹ Bone samples (i.e., rib bone samples and coccygeal vertebrae) were dried (105 C for 8 h) then ether extracted in a soxhlet apparatus (Fick et al., 1979); mineral content (Table 6) and bone ash were subsequently determined.

¹ Mettler Instruments Corporation, Density Determination Kit ME-40290, Hightstown, NJ.

Reference material (e.g., tomato leaves, orchard leaves, bovine liver, milk and serum) from the National Bureau of Standards¹ was included as an internal standard with all samples analyzed for mineral content. All minerals were analyzed by flame atomic absorption spectrophotometry (AAS)² (Perkin-Elmer Corp., 1982) except for cobalt and molybdenum which were analyzed by flameless AAS³ (Perkin-Elmer Corp., 1984) and P which was analyzed colorimetrically using the procedure described by Harris and Popat (1954).

Statistical Analyses

Third Metacarpal, Rib Breaking Load, Coccygeal Vertebrae, Animal Tissues, Ruminal and Abomasal Contents and Rumen Fluid Data

Paired t-tests (Montgomery, 1976) were initially performed to determine if chemical and physical properties of right and left McIII and breaking load of right and left 12th ribs were similar in pairs of bones. Once determined that sides were similar within an animal, these values were averaged and this value used in subsequent statistical analysis.

Third metacarpal, 12th rib breaking load, coccygeal vertebrae, animal tissue, ruminal and abomasal contents and rumen fluid data

¹ National Bureau of Standards, Washington, DC.

² Perkin-Elmer Corporation, Model 5000 with AS-50 auto-sampler, Norwalk, CT.

³ Perkin-Elmer Corporation, Zeeman/3030 with AS-60 auto-sampler, Norwalk, CT.

were statistically analyzed by least squares analysis of variance using the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS, 1982). A completely randomized design (Cochran and Cox, 1957) was used with the basic model:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where

Y_{ij} = jth response of ith treatment

μ = overall mean

T_i = fixed effect of ith treatment

ϵ_{ij} = random component of error, $\sim$ NID (0, σ^2)

Error was assumed to be normally and independently distributed with a mean of zero and variance σ^2 . Correlation coefficients of relationships between various McIII properties were estimated using the PROC CORR procedure of SAS (1982). Graphical representation of relationships between various McIII properties were generated using the PROC GPLOT procedure of SAS (1981); related linear regression equations were determined using the PROC REG procedure of SAS (1982).

Heifer Growth, Ovarian Palpation, Blood, Cow/Calf
Parturition/3 wk Postpartum Parameters, Rib Biopsy,
Feces, Hair and Saliva Data

Data in this section were collected from experimental animals on two or more occasions indicating data analysis for repeated measures of animal parameters was appropriate (Gill and Hafs, 1971). These data were analyzed using a split-plot design (Snedecor and Cochran, 1967) with nesting and cross-classification by least squares analysis

of variance using the PROC GLM procedure of SAS (1982). The basic model used was as follows:

$$Y_{ijkl} = \mu + T_i + A(T)_{j(i)} + P_k + TP_{ik} + \epsilon_{ijkl}$$

where

Y_{ijkl} = lth response of kth period of ith treatment of the jth animal

μ = overall mean

T_i = fixed effect of ith treatment

$A(T)_{j(i)}$ = random effect of jth animal within treatment i

P_k = fixed effect of kth period

TP_{ik} = fixed joint effect of ith treatment and kth period

ϵ_{ijkl} = random component of error, $\sim$ NID (0, σ^2)

As a result, treatment effects were tested using animal within treatment mean square as an error term. Period and period*treatment effects were tested using the error mean square (residual).

Temporal trends were tested for blood and palpation data (linear, quadratic or cubic) as well as for hair and feces data (linear or quadratic). Time trends were not tested for data having only two collection periods (i.e., rib biopsy data, cow/calf parturition/3 wk postpartum blood and milk data and saliva data); in these cases comparisons between periods were made.

Heifer average daily gain and total gain over 210 d ad libitum feeding period, calf birth weights and calf weight change from

parturition to 3 wk postpartum were analyzed using the PROC GLM procedure of SAS (1982).

CHAPTER FOUR
INFLUENCE OF DIETARY PHOSPHORUS LEVEL ON GROWTH
AND REPRODUCTION OF GROWING BEEF HEIFERS

Introduction

Phosphorus (P) has been reported to have more known functions than any other mineral element in the animal body. The importance and essentiality of P in ruminant diets worldwide is well established and is probably the nutrient most frequently given as a supplement to grazing ruminants (Cohen, 1980).

Phosphorus deficiency which is predominantly a condition of grazing cattle (McDowell, 1976; Underwood, 1981) has been the focus of a great deal of research over the past 60 yr. Almost every phase of performance in relation to beef cattle production has been reported to be depressed by a deficiency of P, such as voluntary feed intake, feed efficiency, growth, body condition and all phases of reproduction.

To date, a considerable volume of research work has been published on the requirements of P in beef cattle nutrition; however, there is no clear consensus on the requirements of this mineral element in ruminants as contradictory reports have continued to appear. Confounding many of the literature reports in regards to P requirements and deficiency is the uncertainty of adequate levels of all other nutrients in these reported studies.

The purpose of the present study was to compare the influence of two dietary levels (.12 and .20%, DMB) of P in a long term study on growth and reproduction in growing beef heifers and evaluate the use of various parameters in their ability to reliably and objectively assess P status of these animals.

Materials and Methods

The duration of the present experiment ranged from 525 to 772 d. The experiment consisted of a P depletion phase (270 d), an adaptation period to the basal P supplementation diet (10 d) and a P supplementation phase (ranging from 245 to 492 d in length; common experimental endpoint was day 245 of P supplementation phase for nonpregnant and 3 wk postpartum for pregnant heifers).

Fifteen weaned Angus heifers, 7 to 8 mo of age, 160 ± 3 kg initially were housed under dry lot conditions on concrete floors in a covered barn and allowed ad libitum intake of a low P diet (Table 2) during the 270 d P depletion phase. Animals were bled periodically (Table 3) by jugular vein puncture¹ according to methods described by Fick et al. (1979).

At the end of this period heifers were allowed a 10 d adaptation period to the basal P supplementation diet (Table 2). In the P supplementation phase of the experiment, heifers (averaging 210 ± 6 kg) were allotted randomly ($7 \text{ animals} \cdot \text{group}^{-1}$) to one of two dietary

¹ Jensen-Salsberg Laboratories, Inc., California Bleeding Needles, Kansas City, MO.

levels of P: 1) continuation of low P (LP) basal diet containing .12% P (dry matter basis, DMB), or 2) high P (HP) diet, consisting of basal diet supplemented to provide .20% total P (DMB) (Table 2). Basal diets fed during the course of the experiment were formulated to be low in P yet provide adequate energy, nitrogen, other minerals and vitamins to promote $.5 \text{ kg} \cdot \text{d}^{-1}$ gain. Calcium to P ratios in the P supplementation phase ranged from 3.1:1 in the HP diet to 5.2:1 in the LP diet (6:1 in P depletion phase).

Heifers were group fed ad libitum their respective treatment diets for the initial 210 d of the P supplementation phase. Feed intake was then restricted in an attempt to limit body condition in order to minimize potential calving difficulties. Throughout this period, feed intake was recorded daily for each group. Feed offered was periodically sampled for chemical analyses. Heifers were weighed at 2 wk intervals at 0800 h prior to feeding.

All heifers were bred naturally via 120 d exposure to a single bull (from day 94 to 214 of the supplementation phase). Ovarian activity of heifers was monitored monthly by rectal palpation from day 85 to 239 of the supplementation phase (6 palpations total). After the final palpation, two heifers from both LP and HP groups were diagnosed as nonpregnant¹ and were slaughtered on day 245 of the supplementation phase.

¹ Upon examination of the reproductive tracts of these heifers obtained at slaughter, one heifer of the LP group was found to be pregnant with a 55 to 60 d conceptus.

Remaining pregnant heifers (5 per group; 10 total) were maintained until 3 wk postpartum at which time they were slaughtered. Calving occurred between day 387 (birth of first calf) and 471 (birth of last calf) of the P supplementation phase. Calves were weighed at day of birth (DOB) and 3 wk of age. Blood samples were obtained from both heifers and calves at birth and 3 wk postpartum. Colostrum samples were taken from heifers within the first 6 h after birth of calves. Milk samples were also collected 3 wk postpartum. Two heifers that lost calves due to death were milked out by hand twice daily (0800 and 1700 h) until 3 wk postpartum.

Heifers were periodically sampled throughout the P supplementation phase. Samples collected included whole blood, serum and plasma for all collections, hair, fecal grab samples, saliva and rumen fluid. Liver (from the right lobule), kidney, heart and longissimus dorsi muscle were collected at slaughter according to procedures described by Fick et al. (1979). Rumen and abomasal contents with fluid component were collected at slaughter by making a small incision through the respective compartments and extruding material from within into plastic bottles.

Hair samples were obtained by clipping a 30 cm² area of each heifer's left flank using electric shears. Fecal grab samples (approximately 100 g) were obtained using plastic rectal palpation gloves while animals were restrained in a squeeze chute. Saliva samples were procured using a modified version of the technique described by Murphy and Connell (1970). Rumen fluid was obtained

with animals restrained in a squeeze chute via a stainless steel Frick speculum which was placed in the mouth through which a stomach tube was passed into the rumen. All samples except hair were stored frozen (-15 C) in plastic containers until subsequent chemical analysis.

Feed samples were processed according to methods described by Fick et al. (1979) and analyzed for mineral content. Crude protein concentrations in feed were determined by measuring total nitrogen, following the method of Technicon Industrial System (1978). Serum, plasma, liver, kidney, heart and longissimus muscle samples were also prepared for mineral analysis as described by Fick et al. (1979).

Fecal grab samples, ruminal and abomasal contents (with fluid component) were dried in a forced-air oven at 60 C for 48 h. These samples were then processed and analyzed for mineral content as described for feed samples (Fick et al., 1979).

Colostrum and milk samples were processed for mineral analyses using a modified version of the method Fick et al. (1979) described for forage. Hair samples were prepared for mineral analyses following methodology of Kiatoko et al. (1982).

One, 2 and 2 ml aliquots of whole blood, rumen fluid and saliva, respectively, were digested in HNO_3 and HClO_4 (70%) (7 and 4, 7 and 4, and 4 and 3 ml of HNO_3 and HClO_4 , respectively, for whole blood, rumen fluid and saliva, respectively). Samples were digested in small mouth 50 ml Erlenmeyer flasks until the solution was clear and dense HClO_4 fumes were evolved. Samples were then refluxed for

30 min. Samples were filtered, brought to constant volume and diluted for subsequent mineral analyses.

Reference material (e.g., tomato leaves, orchard leaves, bovine liver, milk and serum) from the National Bureau of Standards¹ was included as an internal standard with all samples analyzed for mineral content. All minerals were analyzed by flame atomic absorption spectrophotometry (AAS)² (Perkin-Elmer Corp., 1982) except for cobalt and molybdenum which were analyzed by flameless AAS³ (Perkin-Elmer Corp., 1984) and P which was analyzed colorimetrically (Harris and Pupat, 1954). Hemoglobin (Hb) was determined colorimetrically⁴ and hematocrit (PCV) by a microhematocrit method.

Animal tissue, ruminal and abomasal contents and rumen fluid data were statistically analyzed using a completely randomized design (Cochran and Cox, 1957). Data were analyzed by least squares analysis of variance using the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS, 1982). Data collected from experimental animals on two or more occasions were analyzed using a split-plot design (Snedecor and Cochran, 1967) as is appropriate for repeated measures of animals (Gill and Hafs, 1971),

¹ National Bureau of Standards, Washington, DC.

² Perkin-Elmer Corporation, Model 5000 with AS-50 auto-sampler, Norwalk, CT.

³ Perkin-Elmer Corporation, Zeeman/3030 with AS-60 auto-sampler, Norwalk, CT.

⁴ Becton-Dickinson Co., Unopette 5857, Rutherford, NJ.

with nesting and cross-classification by least squares analysis of variance using the GLM procedure of SAS (1982). Treatment effects were tested using animal within treatment mean square as an error term. Period and period*treatment effects were tested using the residual mean square as an error term.

Temporal trends were tested for blood and palpation data (linear, quadratic or cubic) as well as for hair and feces data (linear or quadratic). Time trends were not tested for data having only two collection periods (i.e., cow/calf parturition/3 wk postpartum blood/milk parameters and saliva data); in these cases only comparisons between periods were made.

Heifer average daily gain (ADG) and total gain over the 210 d ad libitum feeding period in the P supplementation phase, calf birth weights, as well as calf ADG and calf weight change from parturition to 3 wk postpartum were also analyzed using the PROC GLM procedure of SAS (1982).

Results and Discussion

Average daily gain of heifers during the P depletion phase was $.19 \text{ kg} \cdot \text{d}^{-1}$. Gains similar to those observed in this phase ($.2 \text{ kg} \cdot \text{d}^{-1}$) have been reported by Teh et al. (1982) when dairy calves were fed a diet containing .16%P. Signs of pica exhibited as excessive wood chewing was observed in all heifers during the P depletion phase; however, this behavior was not seen during the P supplementation phase in either treatment group.

Average weight of heifers in both treatment groups at 14 d intervals over the 210 d ad libitum feeding period during the P supplementation phase is shown in Figure 6. After receiving P supplemented diet for 28 d, HP heifers showed an advantage in average weight gain over unsupplemented controls, which was maintained for the duration of this 210 d period. Decrease in average body weight of heifers seen in both treatment groups from day 127 to 140 of the P supplementation phase was likely associated with the introduction of the bull.

Total gain (kg) and ADG ($\text{kg} \cdot \text{d}^{-1}$) were greater ($p < .01$) for HP than LP heifers (Table 7) over the 210 d ad libitum feeding period of the P supplementation phase. The total gain and ADG values represent a 20% benefit to P supplemented heifers. The HP heifers consumed numerically more feed (11%) and also tended to be more efficient (10%) than LP heifers (Table 7). These results are in accord with those of Long et al. (1956) who reported linear increases in feed intake and weight gains over the range of .07, .11 and .15% dietary P in yearling steers. Similarly, Tillman et al. (1959) reported linear increases in weight gain, feed consumption and feed efficiency as dietary P increased from .14 to .20%. Wise et al. (1958) reported that at P levels below .18%, feed consumption and growth were depressed in calves. These animals also exhibited rough hair coats and were unthrifty as compared to calves fed diets containing more than .22%P. Kleiber et al. (1936) explained that P deficiency lowers total efficiency mainly by lowering appetite and secondly, by

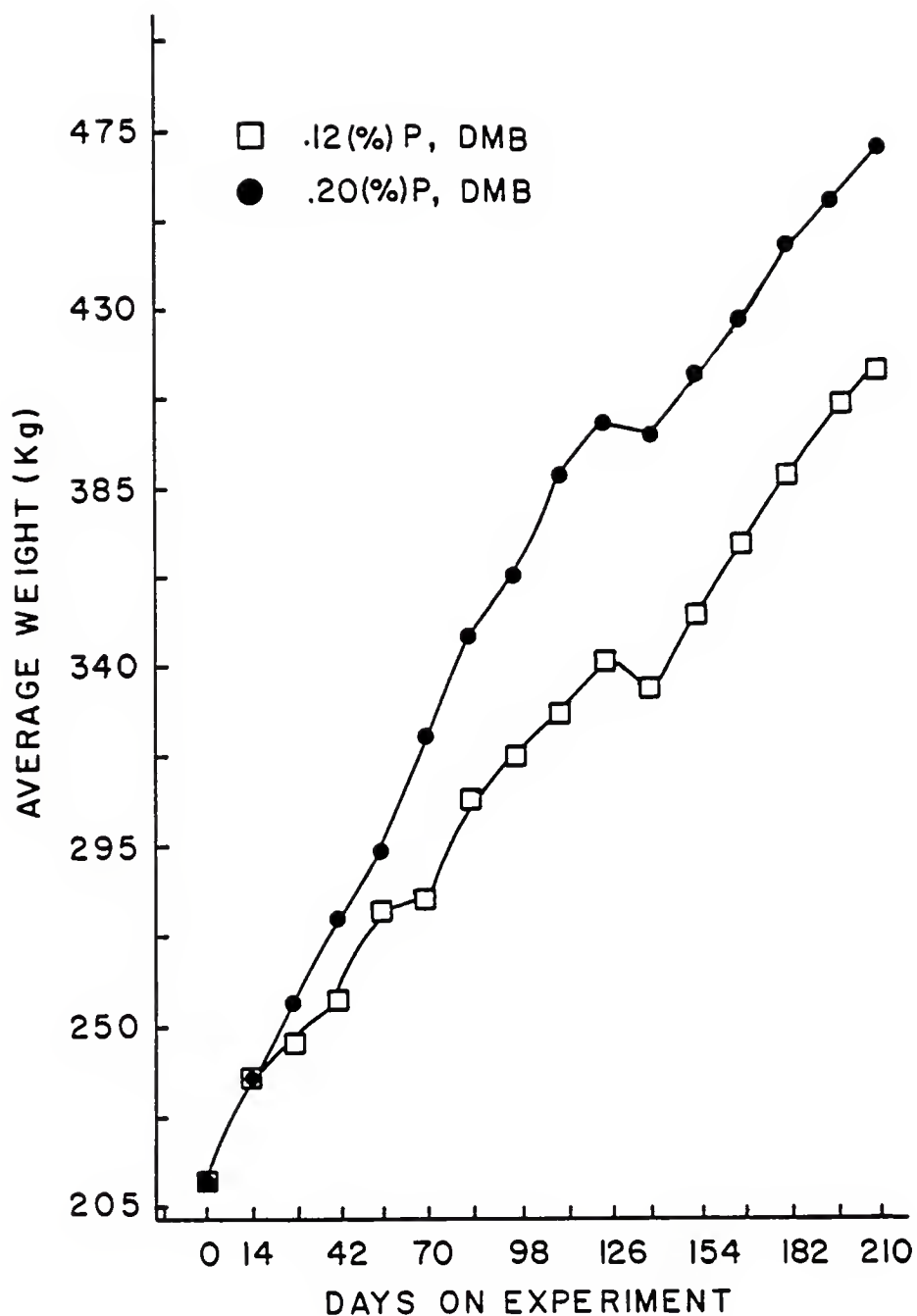


Figure 6. Average body weights of heifers in the unsupplemented P group (.12%P, DMB) and P supplemented group (.20%P, DMB) over the 210 d ad libitum feeding period during the P supplementation phase of the experiment.

Table 7. Influence of dietary phosphorus level on total gain, average daily gain, total dry matter intake and feed:gain of heifers during 210 d ad libitum feeding period of phosphorus supplementation phase.

Item ^b	Dietary Phosphorus Level ^a		SEM ^c
	LP	HP	
Total Gain ^d (kg)	205	257	10.4
Average Daily Gain ^d (kg·d ⁻¹)	.98	1.22	.051
Total Dry Matter Intake (kg)	15089	16973	--
Feed:Gain	10.51	9.44	--

^a LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^b Total gain and average daily gain means based on 7 animals per treatment group over the 210 d ad libitum feeding period. Total dry matter intake represents feed intake over the entire 210 d ad libitum feeding period. Feed:Gain represents total dry matter feed intake for the 210 d ad libitum feeding period divided by total group gain for this period.

^c Standard error of the mean.

^d LP and HP means differ ($p < .01$).

lowering the availability of metabolizable energy for net energy purposes.

Contradictory results to those reported herein have also been published. Henderson and Weakley (1930) reported that dairy calves fed .13%P with an adequate level of Ca grew as well as calves fed .22%P. Beef cattle receiving only *Stylosanthes humilis* (.12%P) had similar dry matter intake and liveweight gain compared to those animals supplemented at the rate of 5 g P·d⁻¹ (Little, 1980). Little (1980) concluded that .12%P (DMB) was adequate for .5 kg·d⁻¹ gain production level. These results were also similar to those of Call et al. (1978) who reported no difference in feed intake, ADG or feed efficiency of Hereford heifers fed either .14 or .36%P (as-fed) over a 2-yr period. In a followup study Butcher et al. (1979) reported that appetite and growth were only affected when dietary P level was further reduced to .09%.

The higher ADG values exhibited during the ad libitum feeding period of the P supplementation phase (i.e., .98 and 1.22 kg·d⁻¹ for LP and HP heifers, respectively) compared to that of the P depletion phase (.19 kg·d⁻¹) are probably associated with such factors as compensatory gain and differences in acceptability in diet formulations between the two experimental phases.

Regression analysis of liveweight gain of heifers during the 210 d ad libitum feeding period of the P supplementation phase is shown in Figure 7. Average gain·animal⁻¹·14 d⁻¹ (kg) and dry matter feed intake·group⁻¹·14 d⁻¹ (kg) during the 210 d ad libitum feeding

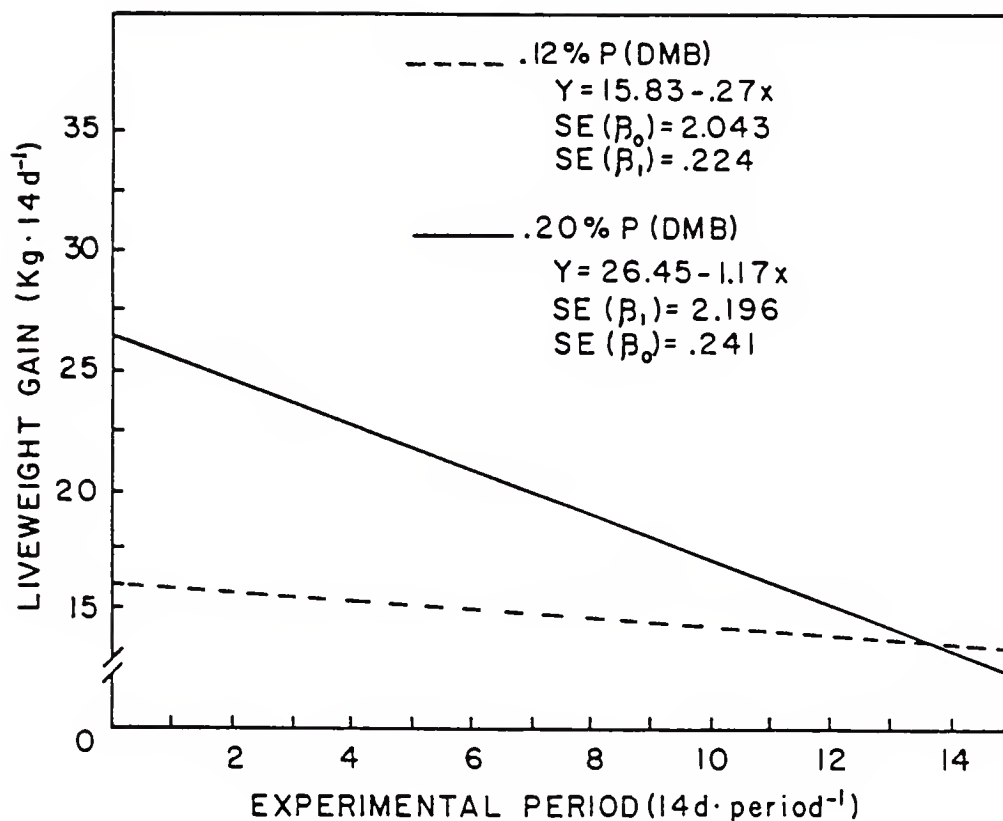


Figure 7. Regression analysis of liveweight gain (Y) and experimental period (X) over the 210 d ad libitum feeding period in the P supplementation phase by treatment group. Each period represents 14 d. Solid line (—) represents P supplemented group (.20%P, DMB), dashed line (---) represents unsupplemented P group (.12%P, DMB). $SE(\beta_0)$ = standard error of intercept estimate; $SE(\beta_1)$ = standard error of slope estimate.

period of the P supplementation phase are shown in Appendix Tables 26 and 27, respectively.

Tables 8 and 9 contain blood serum, plasma and whole blood P and Ca concentrations as well as Hb and PCV values of heifers during the P depletion and supplementation phases, respectively. During the 270 d P depletion phase blood serum inorganic P concentrations showed no temporal trend ($p>.10$). Mean values ranged from 3.41 to 6.21 mg P·dl⁻¹ (Table 8); however, the higher value was based on only 4 observations. Serum Ca concentration showed a cubic trend ($p<.05$). Blood plasma P and Ca concentrations both exhibited cubic period effects ($p<.01$) over the periods it was sampled during the P depletion phase. Whole blood Ca and P concentrations were only obtained twice during the P depletion phase; Ca concentration showed a linear decrease ($p<.01$) from day 180 to 220 while no period effect ($p>.10$) was observed for whole blood P concentration.

Hemoglobin values were similar during all sampling periods of the P depletion phase and exhibited no time trends ($p>.10$). Mean hemoglobin values for periods ranged from 11.28 to 11.81 g·dl⁻¹ (Table 8). Packed cell volume (hematocrit) values fluctuated from 30.0 to 32.4 during the P depletion phase (cubic effect; $p<.10$).

Blood inorganic P concentrations observed in heifers during the P depletion phase (Table 8) are lower than those reported by Little (1980) fed a similar dietary P level. Thompson (1978) indicated that usually the first evidence of P deficiency is a drop

Table 8. Blood serum, plasma and whole blood P and Ca concentrations, hemoglobin and packed cell volume--phosphorus depletion phase.

Item	Sampling Period ^a					SEM ^c
	12/20/82(66) ^b	2/16/83(124)	4/13/83(180)	5/25/83(222)	7/6/83(264)	
Blood Serum (mg·dl ⁻¹)						
P	4.13	4.02	3.41	6.21	3.99	.223
Ca ^h	10.27	10.03	12.15	11.14	12.59	.195
Blood Plasma (mg·dl ⁻¹)						
P ^g	-	3.47	2.58	5.39	3.60	.204
Ca ^g	-	10.09	11.60	10.19	11.75	.163
Whole Blood (mg·dl ⁻¹)						
Ca ^f	-	-	11.94	11.66	-	.306
P	-	-	8.15	7.61	-	.021
Hb ^d (g·dl ⁻¹)	11.52	11.81	-	11.75	11.28	.273
PCV ^e (%) ⁱ	31.7	30.0	-	32.4	31.5	.69

Table 8--continued.

- a Least square means are based on the following number of samples per period: serum Ca and P--14, 10, 10, 4 and 14, respectively, for day 66, 124, 180, 222 and 264; plasma Ca and P--0, 10, 10, 4 and 14, respectively, for day 66, 124, 180, 222 and 264; whole blood Ca and P--0, 0, 10, 4 and 0, respectively, for day 66, 124, 180, 222 and 264; Hb and PCV--7, 10, 0, 12 and 14, respectively, for day 66, 124, 180, 222 and 264.
- b Day of P depletion phase.
- c Standard error of least square mean.
- d Hemoglobin.
- e Packed cell volume.
- f Linear period effect ($p<.01$).
- g Cubic period effect ($p<.01$).
- h Cubic period effect ($p<.05$).
- i Cubic period effect ($p<.10$).

Table 9. Influence of dietary phosphorus level and sampling period on blood serum, plasma and whole blood P and Ca concentrations, hemoglobin and packed cell volume--phosphorus supplementation phase.

Item	Sampling Period ^a										SEM ^d
	10/8/83(79) ^b		11/18/83(120)		1/22/84(186)		3/15/84(238)		5/10/84(294)		
	LP ^c	HP ^c	LP	HP	LP	HP	LP	HP	LP	HP	
Blood Serum (mg·dl ⁻¹)											
pg, i, k	5.79	6.18	2.33	6.24	3.82	4.80	4.96	5.59	4.45	4.74	.287
Ca ^j	9.85	10.32	11.07	9.47	10.42	8.77	8.70	8.28	8.80	8.59	.207
Blood Plasma (mg·dl ⁻¹)											
pg, j, l	3.72	3.86	2.01	5.42	4.10	5.05	4.77	4.84	3.64	4.18	.214
Ca ^h	11.22	11.80	11.05	9.68	9.75	8.27	8.28	7.88	8.50	7.97	.213
Whole Blood (mg·dl ⁻¹)											
p ^h	10.47	11.12	10.55	14.10	18.20	15.30	10.30	11.20	10.50	9.89	.926
Ca ⁱ	8.62	8.25	7.14	6.93	8.54	6.91	6.84	6.53	7.98	7.27	.225
Hb ^e (g·dl ⁻¹) ⁱ	15.6	15.5	14.1	13.5	15.4	15.6	14.9	14.7	12.7	14.1	.35
PCV ^f (%) ^{i, l}	36.4	33.6	39.8	36.9	39.1	40.1	37.9	37.8	37.3	39.1	.70

Table 9--continued.

- a Least square means are based on the following number of samples: serum, plasma, hemoglobin and packed cell volume--7 and 7 for LP and HP groups, respectively, for all periods except May 10, 1984, which was based on 5 and 5 samples for LP and HP groups, respectively; whole blood--7 and 7, 6 and 7, 7 and 7, 7 and 6, and 5 and 5 for LP and HP groups, respectively, for the five periods chronologically.
- b Day of P supplementation phase.
- c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).
- d Standard error of least square mean.
- e Hemoglobin.
- f Packed cell volume.
- g Treatment effect ($p < .01$).
- h Quadratic period effect ($p < .01$).
- i Cubic period effect ($p < .01$).
- j Cubic period effect ($p < .05$).
- k Treatment*period interaction ($p < .05$).
- l Treatment*period interaction ($p < .10$).

in plasma inorganic P below the normal values of 4 to 6 mg P·dl⁻¹ in adult cattle and 6 to 8 mg·dl⁻¹ for young animals. Murray et al. (1936) suggested that blood inorganic P levels indicating P deficiency are less than 3.8 mg·dl⁻¹ serum in mature cattle and 5.3 mg P·dl⁻¹ serum in young cattle. Cunha et al. (1964) suggested that cows with concentrations less than 5 mg P·dl⁻¹ serum were deficient.

No blood serum or plasma mineral concentrations were obtained prior to allowing heifers access to the P depletion diet; however, heifers could have been considered borderline P deficient during this time based on previously stated values in all but one sampling period (day 222). However, mean whole blood P concentration during this period (11.66 mg·dl⁻¹) was considerably lower than the 35 to 45 mg P·dl⁻¹ whole blood reported by Rogers (1975) as normal for cattle, yielding conflicting results to those obtained for blood serum and plasma inorganic P concentration.

Also complicating the use of blood inorganic P concentration in determining P status is the "adjustment" to low dietary P which leads to normal blood inorganic P concentration. Bone is mobilized to maintain a normal blood inorganic P level in P deficient animals; however, mobilization is not rapid (Duncan, 1958); therefore, rapid changes in dietary P may be reflected in blood whereas P deficiency, caused by prolonged low levels of dietary P, may be better detected by bone.

The usefulness of inorganic P in blood serum or plasma is still a matter of contention in regard to it being an adequate criterion for assessing P status in ruminants. Underwood (1966; 1981) and

Conrad (1978) have indicated the adequacy of serum inorganic P as a satisfactory criterion for assessing P status in ruminants, whereas the NCMN (1973) does not recommend its use in this capacity due to its great variation and the poor understanding of the factors that cause this variation. Some of these factors include exercise (Eckles et al., 1930; Garther et al., 1965) or excitement (Eckles et al., 1932) of cattle prior to bleeding, increased storage time or temperature postsampling (Burdin and Howard, 1963), water restriction (Rollison and Bredon, 1960) or time of sampling (Perge et al., 1983) may increase blood inorganic P concentration.

Similar controversy over the use of whole blood P concentration as a P status indicator in ruminants also exists. Call et al. (1978) have suggested that use of whole blood P concentration permits a more comprehensive value for circulating levels of P on a high or low P diet, whereas Miller (1983) has stated that total plasma P (i.e., inorganic P plus organically bound P) is not a very sensitive measure of P deficiency as the organically bound fraction is unavailable to the animal.

Mean blood serum inorganic P concentration displayed a treatment*period interaction ($p < .05$) during the P supplementation phase (Table 9). Mean serum P concentrations were similar after 79 d on treatment diets (i.e., 5.79 vs 6.18 mg P·dl⁻¹ for LP and HP heifers, respectively); however, after 120 d of treatment, LP heifers blood serum inorganic P concentration decreased to 2.33 vs 6.24 mg P·dl⁻¹ for the HP heifers. Blood serum inorganic P (as well as plasma inorganic P and whole blood P) results during this sampling

period agreed with rib bone biopsy P concentration results (Chapter 5) in ranking treatment groups as to level of dietary P. However, by the final collection prior to parturition (d 294 of the P supplementation phase), both groups again exhibited similar serum, plasma and whole blood P concentrations (Table 9) which was not consistent with respect to P content of rib which clearly separated the treatment groups during this period (Chapter 5).

Blood plasma inorganic P concentration followed a similar pattern as observed for serum inorganic P concentrations, except that values tended to be slightly lower than for serum (Table 9). Mean serum Ca concentration was different ($p < .01$) between treatment groups and also showed a cubic period effect ($p < .01$), whereas mean plasma Ca concentration exhibited no treatment effect ($p > .10$) but did show a quadratic time response ($p < .01$). All mean serum and plasma Ca values were within the normal range of 9 to 11 $\text{mg} \cdot \text{dl}^{-1}$ for mature ruminants (Underwood, 1981).

Mean whole blood P concentration was not affected by dietary P level ($p > .10$) but did show a quadratic time response ($p < .01$). It is interesting to note that the mean whole blood P concentration of LP heifers was numerically greater than that of HP heifers on days 186 and 294 when both mean serum and plasma P concentration of LP heifers were less than that of HP heifers (Table 9). This is also surprising since mean PCV values of HP heifers were higher than those of LP heifers at these times. Cubic time trends ($p < .01$) were observed for whole blood Ca concentration during the P supplementation phase.

Hemoglobin displayed cubic time trends ($p < .01$) but was not affected by dietary P level ($p > .10$). Hemoglobin values of both LP and HP heifers during the P supplementation phase were numerically greater than those observed during the P depletion phase. Normal Hb values in adult ruminants are reported to be approximately $12 \text{ mg} \cdot \text{dl}^{-1}$ (Frandsen, 1974).

A treatment*sampling period interaction ($p < .10$) was observed for packed cell volume. As with Hb, PCV values were numerically greater in the P supplement phase than in the P depletion phase. Frandsen (1974) indicated PCV values around 40 were considered normal in adult ruminants.

Even though great care was taken in obtaining all blood samples--considerable variation (Tables 8 and 9) was observed with respect to P concentration in serum, plasma and whole blood. This would indicate that great care should be taken when interpreting data as to P status in cattle when using any of these criteria as the sole indicator of P status (as was evidenced in blood P and bone P discrepancy on d 294).

Dietary P level had no effect ($p > .10$) on liver, kidney, heart or muscle P concentration (Table 10). Ruminal and abomasal contents P concentration likewise showed no treatment effect ($p > .10$); however, HP heifers had numerically greater P concentrations for both samples than LP heifers (.61 vs .49% and .49 vs .35% for ruminal and abomasal contents, respectively; Table 10).

Dietary P level also had no effect ($p > .10$) on rumen fluid P concentration in the present study. There was great variability

Table 10. Influence of dietary P level on selected tissues, ruminal contents, abomasal contents and rumen fluid phosphorus concentration.

Item ^{b,c,d}	Dietary Phosphorus Level ^a		SEM ^e
	LP	HP	
Liver, %P	.66	.66	.023
Kidney, %P	.73	.76	.040
Heart, %P	.80	.73	.030
Muscle, %P	.50	.44	.041
Rumen contents, %P	.49	.61	.064
Abomasal contents, %P	.35	.49	.071
Rumen fluid, $\mu\text{g P}\cdot\text{ml}^{-1}$	272.6	274.9	57.10

^a LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^b All values on a dry matter basis except rumen fluid ($\mu\text{g P}\cdot\text{ml}^{-1}$).

^c Least square means are based on 7 animals per treatment for all items except for liver biopsy (7 and 6 for LP and HP groups, respectively) and ruminal and abomasal contents (5 and 5 for LP and HP groups, respectively).

^d Rumen fluid was obtained on day 294 of the P supplementation phase; all other items obtained at slaughter.

^e Standard error of least square means.

between animals within treatment groups with respect to P concentration of rumen fluid. Values ranged in LP heifers from 61.6 to 413.4 $\mu\text{g P}\cdot\text{ml}^{-1}$ and from 104.5 to 525.9 $\mu\text{g P}\cdot\text{ml}^{-1}$ in HP heifers. Mean rumen fluid P concentrations reported herein are similar to those reported by Cohen (1978, cited in Cohen, 1980) of 150 and 183 $\text{mg P}\cdot\text{L}^{-1}$ in heifers with low intakes of dietary P (5.0 and 15.3 $\text{g}\cdot\text{d}^{-1}$, respectively).

Mean fecal, hair and saliva P concentrations are shown in Table 11. Fecal P levels numerically ranked the treatment groups as to P intake; however, no statistical differences ($p>.10$) were observed over the three sampling periods. Clark et al. (1973) indicated that since P homeostasis in ruminants is achieved to a great extent in the gastro-intestinal tract by controlling secretion and reabsorption of salivary P, fecal losses should reflect intake or absorption. Cohen (1974) reported P intake of cattle may be estimated from regression equations of the intake of a specific feed source on total daily P excretion.

However, problems associated with the use of fecal P concentrations to assess P status in ruminants include selective grazing behavior and plant species varying in P digestibility and availability (Cohen, 1974). Also, large differences in feed intake (Read et al., 1986a, b) may result in total fecal output differing between P supplemented and unsupplemented groups, resulting in a difference in total mass of P excreted, but with no apparent difference in %P in the sample. Fecal P concentrations in the

Table 11. Influence of dietary phosphorus level and sampling period on fecal, hair and saliva phosphorus concentration.

Item ^c	Sampling Period ^a						SEM ^e
	11/18/83 (120) ^b		3/15/84 (238)		5/10/84 (294)		
	LP ^d	HP ^d	LP	HP	LP	HP	
Feces (%P)	.39	.48	.35	.52	.39	.48	.040
Hair ($\mu\text{g P}\cdot\text{g}^{-1}$)	152.3	164.7	153.7	154.0	170.5	160.3	9.72
Saliva ^f ($\text{mg P}\cdot\text{dl}^{-1}$)	--	--	29.5	35.3	25.4	37.0	5.05

^a Least square means are based on the following number of samples: feces--7 and 5, 6 and 7, 5 and 5 for the LP and HP groups, respectively, for the three collections chronologically, respectively; hair--6 and 7, 7 and 7, 5 and 5 for the LP and HP groups, respectively, for the three collections chronologically, respectively; saliva--7 and 7, 5 and 5 for the LP and HP groups, respectively, for the two collection chronologically, respectively.

^b Day of P supplementation phase.

^c Feces and hair P concentrations are on dry matter basis.

^d LP (low phosphorus; .12%P, DMB); HP (high phosphorus, .20%P, DMB).

^e Standard error of least square mean.

^f Treatment effect ($p < .05$).

present experiment are similar to those reported by other groups (Cohen, 1974; Judkins et al., 1985; Read et al., 1986a).

Dietary P level had no affect ($p>.10$) on hair P concentrations (Table 11). The HP heifers showed a slight advantage over the first two sampling periods; however, the reverse of this situation was observed during the third collection. The latter finding is inconsistent with blood serum and plasma P concentrations as well as P concentration of 12th rib bone biopsies (Chapter 5) obtained on the same sampling date. This perhaps strengthens the conclusion of Cohen (1973a, b) that hair P is not a satisfactory indicator of P status in cattle, in contrast to the findings of Anke (1967) and Legel (1971) who reported hair P concentration to be a suitable indicator of P status in ruminants.

Saliva P levels were greater ($p<.05$) in HP than LP heifers (Table 11). Values reported herein are considerably higher than those reported recently by Judkins et al. (1985). Clark (1953) reported salivary P concentration of P deficient animals to be approximately 50% that of animals supplemented with P. Judkins et al. (1985) indicated that since P is cleared from blood in ruminants primarily by the salivary glands, salivary P concentration may be indicative of blood levels and recycling. However, these authors suggested that salivary P concentration may not be a reliable indicator of P status due to variation in P concentration caused by varying amounts of saliva being secreted daily by cattle. Perge et al. (1983) found wide variation in salivary P concentration as affected by dietary supply of Ca and P as well as time of sampling.

Table 12 shows the influence of dietary P and palpation date on ovarian volume estimated by rectal analysis. Only the first four of the six palpations were used in statistical analysis as the final two palpations were performed strictly for pregnancy diagnosis. Heifers were 18 to 19 mo of age at the time of first palpation (October 14, 1983). There was no dietary P level*palpation date interaction ($p>.10$). Dietary P level also had no affect ($p>.10$) on either right, left or total ovarian volume (i.e., right plus left ovarian volume), whereas time responses were found for right (linear trend; $p<.01$), left (quadratic trend; $p<.05$) and total ovary volume (cubic trend; $p<.01$).

Prior to the initial palpation, no normal estrous behavior was observed in heifers of either treatment group. Only 1 of 14 heifers (the lone heifer being from HP group) was found to possess a corpus luteum (CL) at the initial palpation. Three heifers in each group were found with 4 mm or larger follicles during this initial palpation. Also, all heifers were regarded as having prepubertal uteri initially, with the exception of the HP heifer with the CL. By the third palpation six heifers in each group were regarded as having normal cycling uterine tone and also possessed CL.

Puberty of heifers can be influenced by many factors including hormones, genetics, environment and nutrition (Sorenson, 1979). Short and Bellows (1971) reported that weight and age greatly affect onset of puberty in heifers. Perhaps the reduced body weight per day of age well into the P supplementation phase had an affect on the low

Table 12. Influence of dietary phosphorus level and time of palpation on right, left and total ovary volume.

Item ^c	Palpation Date							
	10/14/83 (85) ^a		11/10/83 (112)		12/16/83 (148)		1/16/84 (179)	
	LP ^a	HP ^a	LP	HP	LP	HP	LP	HP
Right Ovary ^f (mm ³)	1937	2959	2560	4738	4062	3403	6841	8775
								1048.3
Left Ovary ^e (mm ³)	1751	1463	4147	3561	2832	4794	6061	5170
								874.2
Total Volume ^g (right plus left) (mm ³)	3688	4422	6707	8299	6894	8197	12902	13945
								1053.3

^a Day of P supplementation phase.

^b LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^c Means based on 7 animals per treatment group (14 total).

^d Standard error of the mean.

^e Linear period effect (p<.01).

^f Quadratic period effect (p<.05).

^g Cubic period effect (p<.01).

sexual activity observed in all heifers (heifers were 210 ± 6 kg and 16 to 17 mo of age at initiation of the P supplementation phase). However, both age and weight of heifers are considered within the range of values (7 to 18 mo of age and 200 to 450 kg) suggested as normal for these parameters for initiation of puberty in heifers (Sorenson, 1979).

Molybdenum (Mo) was added to the basal diet (Table 2) at the rate of $5 \text{ mg} \cdot \text{kg}^{-1}$ (dry basis). Phosphorus depletion and supplementation basal diets analyzed 4.30 and $4.70 \text{ mg Mo} \cdot \text{kg}^{-1}$, respectively (Table 2). Phillippo et al. (1984) recently reported on Mo induced infertility and low conception rates in cattle fed $5 \text{ mg Mo} \cdot \text{kg}^{-1}$ diet dry matter. These authors reported that low Cu status induced by high Mo levels was associated with depressed growth rate and impaired reproductive performance; however, they point out that altered secretion of luteinizing hormone suggests that Mo may have an early effect on the hypothalamic-pituitary axis. Copper levels in the present trial, 13.8 and $14.9 \text{ mg} \cdot \text{kg}^{-1}$ for the basal P depletion and supplementation diets, respectively, were above recommended levels of $8 \text{ mg} \cdot \text{kg}^{-1}$) (NRC, 1984) and may have provided some protection against high dietary Mo levels.

Five of seven heifers in the HP group were found to be pregnant and six of seven in the LP group. Birth weight of calves were greater ($p < .10$) from HP than LP dams (Table 12). One calf was born stillborn in the HP group and another HP calf died during the 21 d postpartum period unrelated to treatments. All five calves born to LP heifers survived the 21 d period following parturition. The HP

Table 13. Influence of dietary phosphorus level on calf birth weight, calf weight change and calf average daily gain from parturition to 3 wk postpartum (21 d period).

Item ^b	Dietary Phosphorus Level ^a		SEM ^c
	LP	HP	
Calf birth weight (kg) ^d	22.7	26.9	1.60
Calf weight change (kg·21d ⁻¹)	12.2	15.7	1.71
Calf average daily gain (kg·d ⁻¹)	.58	.75	.080

^a LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^b Least square means are based on the following number of animals per treatment group: 5 and 5 for LP and HP groups, respectively, for calf birth weight; 5 and 3 for LP and HP groups, respectively, for calf weight change and calf average daily gain over the 21 d period from day of birth to 3 wk postpartum.

^c Standard error of least square mean.

^d LP and HP means differ ($p < .10$).

calves gained more weight (Table 12) numerically than LP calves (15.7 vs 12.2 kg, respectively) over the 21 d postpartum period; however, the HP least square mean is based on only 3 calves. Similarly, calf ADG was numerically greater for HP calves (Table 12). This could possibly be related to increased milk yield from HP heifers; however, this variable was not quantified.

There appears to be wide variation in defining minimum P requirements for the various phases of reproduction in female beef cattle. Phosphorus deficiency is accepted as a major nutritional factor depressing reproductive efficiency in mammals. In some studies, ovarian function and fertility of bovine females appear to be quite sensitive to P intake (Eckles et al., 1926; Theiler et al., 1928; Hignett, 1950; Hignett and Hignett, 1951; O'Moore, 1952), whereas other work has failed to show a diminished reproductive performance of animals on low P diets (Guilbert and Goss, 1932; Huffman et al., 1933; Eckles et al., 1935; Palmer et al., 1941; Holzschuh et al., 1971; Call et al., 1978; Butcher et al., 1982; Greene et al., 1985; Judkins et al., 1985; Call et al., 1987). Results of the present experiment would suggest agreement with the latter proposition that low dietary P per se did not affect reproductive performance.

Tables 14 and 15 show cow and calf blood serum, plasma and whole blood P and Ca concentrations as well as Hb and PCV values at day of birth and 3 wk postpartum, respectively.

Table 14. Influence of dietary phosphorus level on cow blood P and Ca concentrations, hemoglobin, packed cell volume and colostrum/milk P and Ca concentrations at parturition and 3 wk postpartum.

Item	Time of Sampling ^a				SEM ^c
	Parturition		3 wk postpartum		
	LP ^b	HP ^b	LP	HP	
Blood Serum (mg·dl ⁻¹)					
p _{g,h}	2.91	4.74	4.05	5.05	.381
Ca ^h	9.81	9.93	9.52	9.50	.170
Blood Plasma (mg·dl ⁻¹)					
p _{g,i}	2.28	4.18	3.55	4.61	.322
Ca	8.95	9.63	8.84	8.98	.280
Whole Blood (mg·dl ⁻¹)					
p _{g,j}	10.17	16.27	12.65	14.87	.983
Ca ^j	6.73	8.05	7.86	7.31	.331
Hb ^d (g·dl ⁻¹) ^f	12.9	11.4	12.1	12.1	.43
PCV ^e (%) ^h	33.6	31.3	31.9	30.2	.64
Colostrum/Milk (%, dry basis)					
P	.88	1.01	.91	1.02	.060
Ca ⁱ	.81	.92	1.08	1.10	.090

^a Means based on 5 animals per treatment group (10 total) for both day of birth and 3 wk postpartum.

^b LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^c Standard error of the mean.

^d Hemoglobin.

^e Packed cell volume.

^f Treatment effect (p<.05).

^g Treatment effect (p<.01).

^h Period effect (p<.10).

ⁱ Period effect (p<.05).

^j Treatment*period interaction (p<.10).

Table 15. Influence of dietary phosphorus level on calf blood P and Ca concentrations, hemoglobin and packed cell volume at day of birth and 3 wk postpartum.

Item	Time of Sampling ^a				SEM ^c
	Day of Birth		3 wk postpartum		
	LP ^b	HP ^b	LP	HP	
Blood Serum (mg·dl ⁻¹)					
P ^j	4.92	5.18	7.39	8.14	.280
Ca ^h	11.67	10.84	10.08	10.17	.461
Blood Plasma (mg·dl ⁻¹)					
P ^j	4.25	3.85	6.78	6.98	.381
Ca ^f	10.60	9.55	9.67	9.72	.487
Whole Blood (mg·dl ⁻¹)					
P ^{g,i}	13.51	18.75	18.33	22.14	1.211
Ca	7.89	8.42	8.35	8.37	.219
Hb ^d (g·dl ⁻¹)	9.14	8.62	9.86	9.59	.458
PCV ^e (%)	29.7	26.8	27.8	26.8	.97

^a Least square means based on the following number of samples: 5 for all LP means, 4 for HP day of birth and 3 for HP 3 wk postpartum.

^b LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^c Standard error of the least square mean.

^d Hemoglobin.

^e Packed cell volume.

^f Treatment effect (p<.10).

^g Treatment effect (p<.01).

^h Period effect (p<.10).

ⁱ Period effect (p<.05).

^j Period effect (p<.01).

Both serum and plasma inorganic P concentration were greater ($p < .01$) in HP heifers as well as being greater ($p < .10$) 3 wk postpartum than at parturition (Table 14). Serum and plasma Ca concentrations were not affected by dietary P level ($p > .10$); serum Ca decreased ($p < .10$) 3 wk postpartum, whereas plasma Ca was not affected by time of sampling ($p > .10$). Both whole blood P and Ca showed significant treatment*sampling period interactions ($p < .10$).

The variability of the whole blood P assay as compared to either serum or plasma inorganic P is seen in Table 14 as standard errors of whole blood P are approximately 3-fold that of serum or plasma P assays. Hemoglobin was greater ($p < .05$) for HP than LP heifers. Packed cell volume was affected by sampling date ($p < .10$) but not by dietary P level ($p > .10$).

Salih et al. (1987) reported that serum Ca and P and PCV were higher at parturition than 3 mo postpartum. This difference with respect to the present trial may be associated with the different time intervals involved in sample collection postpartum. Read et al. (1986a) have reported results similar to those in the present trial.

Calf serum and plasma inorganic P concentrations were not affected by dietary P level of the dam ($p > .10$; Table 15). Serum and plasma P concentrations increased ($p < .01$) from day of birth to 3 wk postpartum. Serum and plasma P concentrations at 3 wk postpartum are typical of those reported as normal for young cattle (Underwood, 1981).

Serum and plasma Ca concentration did not act in a similar pattern. Serum Ca was not affected by dietary P level of the dam

($p > .10$), but decreased ($p < .10$) from day of birth to 3 wk postpartum. Conversely, plasma Ca was not affected by sampling date ($p > .10$) but was influenced by dietary P level of the dam ($p < .10$; Table 15).

Calf whole blood P was affected by both dietary P level of the dam ($p < .01$) and sampling period ($p < .05$). There was no treatment*sampling date interaction for this variable (Table 15). The pattern of increasing P concentration in the calf from day of birth to 3 wk postpartum was similar for blood serum, plasma and whole blood. Calf whole blood Ca hemoglobin and PCV were not affected by either dietary P level of dam ($p > .10$) or sampling period ($p > .10$).

The P and Ca content of colostrum and milk of heifers at parturition and 3 wk postpartum are shown in Table 14. Dietary P level had no effect on P or Ca content of colostrum or milk (dry basis) ($p > .10$); however, HP heifers had a numerically greater P and Ca content in both fluids. Forar et al. (1982) reported that milk inorganic P was not affected by dietary P or Ca, but that it did decline as lactation proceeded and as milk yields decreased. Milk yield was not measured in the present experiment. When expressed on a dry matter basis, there was also no difference ($p > .10$) between sampling periods in P content (i.e., similar content between colostrum and milk), whereas Ca content was greater ($p < .05$) 3 wk postpartum. Salih et al. (1987) reported that P and Ca content of colostrum for Brahman cattle was greater ($p < .01$) than milk samples collected 3 mo postpartum.

Conclusions

Results of the present study would suggest .12%P (DMB) does not optimize growth response in young growing heifers as heifers fed .20%P (DMB) outgained ($p < .05$) heifers that received the lower P level during the 210 d ad libitum feeding period of the P supplementation phase. Heifers receiving the .20%P level also tended to consume more feed and be more efficient in its utilization.

Although P insufficiency has been reported to lower reproductive performance in beef cattle, in the present study .12%P was not detrimental to onset of puberty or conception rate. Results herein may suggest that for a limited time, .12%P is adequate to maintain reproductive performance in young heifers, relative to the .20% dietary P level.

Results of blood P analyses generally tended to rank treatment groups as to dietary P level; however, due to variability associated with blood P analysis, confidence in discerning P status would be difficult if blood P was used as a sole indicator. For the majority of sampling periods both LP and HP groups were able to maintain normal serum and plasma P concentrations. Results of 12th rib bone biopsy (Chapter 5) yielded much clearer results at any given sampling time as to P status; however, this experiment represents a special situation in which the dietary regimen was constant throughout, unlike the situation associated with grazing ruminants.

Fecal and hair P levels proved insensitive for distinguishing between LP and HP heifers. Saliva P concentration ranked treatment

groups in both sampling periods relative to dietary P level; however, caution in using this method to assess P status is advised due to a high risk of possible contamination.

CHAPTER FIVE
CHEMICAL AND PHYSICAL PROPERTIES OF BONE IN
BEEF HEIFERS AS INDICATORS OF PHOSPHORUS STATUS

Introduction

Among the most important requirements of any animal is the development of an adequate skeletal support system. Interest in studying chemical, physical and structural properties of bone as they relate to bone development has intensified. Clearly this is demonstrated in livestock operations where the trend for maximum production has created situations where optimal bone development and strength are necessary components of animal performance. This is true not only in young animals, but also in regard to extending the useful life of animals within the breeding herd.

Bone development along with the chemical and physical properties of the material is affected by a number of factors including age, nutrition, hormonal influences and disease. Dietary P level has been shown to greatly affect bone development and associated chemical and physical properties in different species (Harmon et al., 1970; Schryver, 1978; Crenshaw et al., 1981b; Little, 1984); however, few comprehensive studies involving the influence of dietary P level on various bone properties in the bovine have been reported.

The objectives of the present experiment were to 1) evaluate the effects of two levels of dietary P on various chemical and physical

properties of several different bones in growing beef heifers and 2) assess the use of different bone properties as indicators of P status.

Materials and Methods

The duration of the present experiment ranged from 525 to 772 d. The experiment consisted of a P depletion phase (270 d), an adaptation period to the basal P supplementation diet (10 d) and a P supplementation phase (ranging from 245 to 492 d in length; common experimental endpoint was day 245 of the P supplementation phase for nonpregnant and 3 wk postpartum for pregnant heifers).

Fifteen weaned Angus heifers, 7 to 8 mo of age, averaging 160 ± 3 kg initially, were housed under dry lot conditions on concrete floors in a covered barn and allowed ad libitum intake of a low P diet (Table 2) during the 270 d P depletion phase. At the end of this period heifers were allowed a 10 d adaptation period to the basal P supplementation diet (Table 2). In the P supplementation phase of the experiment heifers (averaging 210 ± 6 kg) were allotted randomly ($7 \text{ animals} \cdot \text{group}^{-1}$) to one of two dietary levels of P: 1) continuation of low P (LP) basal diet containing .12% P (dry matter basis, DMB), or 2) high P (HP) diet, consisting of basal diet supplemented to provide .20% total P (DMB) (Table 2). Basal diets fed during the course of the experiment were formulated to be low in P yet provide adequate energy, nitrogen, other minerals and vitamins to promote $.5 \text{ kg} \cdot \text{d}^{-1}$ gain. Calcium to P ratios ranged from 3.1:1 in the HP diet to 5.2:1 in the LP diet (6:1 in the P depletion phase).

Animals in this second phase of the experiment were group fed ad libitum their respective diets for the initial 210 d of the P supplementation phase. Feed intake was then restricted in an attempt to limit body condition in order to minimize potential calving difficulties.

All animals in both treatment groups were bred naturally via 120 d exposure to a single bull. Two heifers from each treatment were determined via rectal palpation as nonpregnant and were subsequently slaughtered on day 245 of the supplementation phase and bone samples recovered. Remaining pregnant heifers (5 per group) were maintained until 3 wk postpartum with bone samples collected at slaughter. Calving occurred between day 386 and 471 of P supplementation phase.

Rib cortical bone biopsies were obtained serially on days 120 and 294 of the P supplementation phase using a modified version of the surgical procedure described by Little (1972b). Biopsy samples were removed from the 12th rib of all animals (left side on d 120 and right side on d 294) using a 1.4 cm diameter trephine. Trephine depth was set to allow the sample collected to include both medial and lateral cortical bone of the intercostal appendage. Samples were immediately wrapped in .9% saline soaked gauze and frozen (-15 C) until further analysis. Upon thawing, a stainless steel scalpel was used to carefully remove bone marrow from the medial side of the rib biopsy sample. Biopsies were then rinsed in deionized water and blotted dry. Rib cortical bone density ($\text{g}\cdot\text{cm}^{-3}$) was determined using

a Mettler density determination kit.¹ Biopsy samples were subsequently dried at 105 C for 8 h then ether extracted in a soxhlet apparatus for 48 h according to the procedure of Fick et al. (1979). Samples were then ashed in a muffle furnace at 600 C for 12 h. Calcium and Mg were analyzed by flame atomic absorption spectrophotometry (AAS)² (Perkin-Elmer Corp., 1982) while P was determined colorimetrically (Harris and Popat, 1954).

At slaughter, tails (with hide removed), left and right third metacarpals (McIII) and left and right 12th ribs (from the transverse lumbar process to the distal articulating cartilaginous ends) were collected, wrapped in .9% saline soaked gauze and frozen (-15 C) until further analysis.

Coccygeal vertebrae V, VI and VII were located in tail samples and removed with a stainless steel scalpel. All soft tissue was removed taking care not to disrupt the mineral fraction. Cartilaginous caps over the proximal and distal vertebral ends were removed prior to analysis. Vertebra bone density ($\text{g}\cdot\text{cm}^{-3}$) and mineral analyses were determined as described previously for rib cortical bone biopsy samples. Bone ash content of whole vertebrae was expressed as g per vertebra and defined as bone mineral content (VBMC).

¹ Mettler Instruments Corporation, Density Determination Kit ME-40290, Hightstown, NJ.

² Perkin-Elmer Corporation, Model 5000 with AS-50 auto-sampler, Norwalk, CT.

Third metacarpals were prepared for mechanical testing in a similar manner to that employed by Lawrence (1986). All soft tissue including fibrous periosterm was removed; care was taken not to scratch the surface of the bones. While dissecting out all surrounding soft tissue, McIII were always kept submerged in .9% saline. Metacarpals were prepared in this manner to ensure that biomechanical tests would be performed on "wet" bones which closely resemble bones as they exist in the animal. Sedlin and Hirsch (1966) reported that as little as 10 min exposure to air can greatly affect the biomechanical properties of bone.

Bone mineral density (WBMD) and bone mineral content (WBMC) of whole wet McIII were determined by dual photon absorptiometry.¹ Cortical bone index (CBI) and radiographic area index (XRI) were determined from anterioposterior radiographs² using the same radiographic guidelines described by Meakim et al. (1981). Calculation of CBI was performed according to a method described by Suttie et al. (1983) and XRI using a method described by Meema et al. (1964).

Mechanical properties of wet McIII were tested by bending by three-point loading using an Instron Floor Model Testing Machine.³ Metacarpals were rested on round pivot supports 13 cm apart. Force

¹ Lunar Radiation Corporation, Bone Mineral Analyzer Dual Photon Rectilinear Scanner Model DP3, Madison, WI.

² Universal X-Ray Products, Easymatic Super 325, Chicago, IL.

³ Instron Corporation, Model 1125, Canton, MA.

was applied to the posterior surface of McIII at a constant rate of $50 \text{ mm} \cdot \text{min}^{-1}$. Testing was completed upon failure of the McIII. Applied loads were normalized on the mid-span geometry of the bone cross-section. A strip chart recorder was utilized to record the force-deformation curve (Figure 3) necessary for calculation of mechanical properties. Breaking load (BL, kg), the force required to cause failure of the McIII, was determined directly from the force-deformation curve.

Broken McIII were placed in 70% ethanol solution (w/v) for 72 h to aid in removing bone marrow. Metacarpals were then carefully reassembled using Elmer's Glue-All.¹ Reassembled McIII were then placed in plastic containers slightly larger than all McIII dimensions and liquid urethane² poured around them. Once solidified, the liquid urethane provided a uniform, flat surface enabling accurate removal of particular diaphysis cross-sections of interest from McIII. A 2 cm cross-section was removed at the point of loading (1 cm on either side of midpoint) on the McIII diaphysis using a table band saw.

Caliper cross-sectional measurements from the 2 cm sections were anteroposterior (AP) bone diameter (B), AP medullary diameter (b), lateromedial (LM) bone diameter (D) and LM medullary diameter (d) (Figure 4). Diameters of cross-sections were used in an equation

¹ Borden Incorporated, Elmer's Glue-All, Columbus, OH.

² Bradson Auto Marine, Incorporated, Brad Foam, Pinellas Park, FL.

$(\pi/64 (BD^3 - bd^3))$ for calculating the area moment of inertia of an ellipse (MIE, cm^4). Caliper measurements were also used to determine the medial-lateral wall thickness (MLWT, mm) using the equation $((D-d)/2)$.

Equations for calculating breaking strength (BS, MPa) and Young's modulus (E, GPa) were $BS = (F)(L)(C)/4(MIE)$ and $E = (F)(L^3)/48(MIE)(D)$, where F = force (kg), L = distance between supports the bone rests upon (cm), C = distance from neutral axis to extreme outer fiber (cm) and D = deformation (cm). Force and deformation values were determined from force-deformation curves. Strain was computed as the change in length per unit length at the point of failure.

Metacarpal cross-sections were photographed along with a reference standard and actual area (PAI, mm^2) irregardless of geometry determined by planimeter.¹ Equations for calculating cross-section area indices (mm^2) for known geometrical shapes were $(B^2 - b^2)$ for circular cross-section area index from bone (CAI) and radiographic area index from AP radiographs (XRI) and $(BD - bd)$ for elliptical area index (EAI) from bone.

The 2 cm cross-sections were dried, ether extracted and ashed as previously described for bone biopsies and coccygeal vertebrae. Metacarpal cross-section bone ash was expressed as $\text{g} \cdot 2 \text{ cm}^{-1}$ section and defined as bone mineral content (BMC). Calcium, Mg and P

¹ Numonics Corporation, Model 1250-1, Lansdale, PA.

analyses of McIII cross-section were performed as for rib bone biopsies and coccygeal vertebrae.

Left and right 12th ribs were prepared for mechanical testing in a similar fashion as the McIII. A 15 cm section was removed from the articulating cartilaginous end of each rib. Rib sections were tested by bending by three-point loading as previously described for McIII. Sections were loaded such that force was applied to the concave (medial) portion of the intercostal curvature. All testing parameters were as for McIII with the exception that the distance between the roller supports was 10 cm. Breaking load (BL) was the only mechanical property determined for the rib bones.

Paired t-tests (Montgomery, 1976) were initially performed to determine if chemical and physical parameters measured in right and left McIII and BL of right and left 12th ribs were similar in pairs of bones. Once determined that sides (i.e., right and left) were similar for these properties within an animal, average side response was computed and used in subsequent statistical analyses.

All data were analyzed by analysis of variance using the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS, 1982). Correlation coefficients between various chemical and physical McIII properties were estimated using the PROC CORR procedure of SAS (1982). Graphical representation of relationships between various McIII properties were generated using the PROC GPLOT procedure of SAS (1981); related linear regression equations were determined using the PROC REG procedure of SAS (1982).

Results and Discussion

Twelfth Rib

Analysis of the skeleton as an index of mineral status has been well established (Duckworth and Hill, 1953; Benzie et al., 1959). Hill (1962) described the difference among bones in the rapidity of bone mineralization and with which bone mineral can be mobilized. Mineral from the ribs and axial skeleton is readily mobilized. Little (1972b) described a biopsy technique for serially sampling bovine rib which would allow changes in mineral status of a given set of animals to be followed. Table 16 shows results of a modified version of this method which was used to study 12th rib cortical bone properties as affected by dietary P level and sampling period.

Dietary P level*sampling period interaction ($p < .05$) revealed that percent ash was different ($p < .01$) between HP and LP heifers during the initial collection (61.9 vs 55.7%, respectively) but not for the second collection. Percent ash increased ($p < .01$) from the first to the second biopsy collection (Table 16). These results may suggest that, as the heifers matured by the second collection (and growth tapered off), more mineral became available for deposition in bone.

Bone density ($\text{g}\cdot\text{cm}^{-3}$) of rib cortical bone was greater ($p < .05$) for the HP group; also, bone density increased ($p < .01$) from the first to second collection (Table 16). A lowered bone density indicates reduced bone mineral content thus causing increased porosity within the bone. Bone density results in this study are consistent with those of Tejada (1984) who concluded that specific gravity (actually

Table 16. Influence of dietary phosphorus level and sampling period on various 12th rib cortical bone parameters.

Item	Sampling Period ^a				SEM ^d
	11/18/83 (120) ^b		5/10/84 (294)		
	LP ^c	HP ^c	LP	HP	
Ash (%) ^{f, j, k}	55.7	61.9	63.1	63.8	.97
Bone density ^{f, j} (g·cm ⁻³)	1.32	1.47	1.50	1.55	.029
P (%) ^f , ash	16.5	16.6	16.8	17.5	.33
Ca (%) ^f , ash	42.9	42.4	42.5	42.3	.93
Mg (%) ^{f, j} , ash	.40	.47	.49	.55	.023
P (mg·cm ⁻³) ^{f, j} , fresh ^{g, j}	123.0	152.0	157.0	174.2	6.92
Ca (mg·cm ⁻³) ^{f, j} , fresh ^{e, j}	320.6	385.5	402.3	417.1	14.88
Mg (mg·cm ⁻³) ^{f, j} , fresh ^{g, j}	3.0	4.3	4.6	5.3	.24

^a Least square means are based on the following number of samples: 14 for November 18, 1983 (7 per treatment group) and 10 for May 10, 1984 (5 per treatment group).

^b Day of P supplementation phase.

^c LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20% P, DMB).

^d Standard error of least square mean.

^e Treatment effect (p<.10).

^f Treatment effect (p<.05).

^g Treatment effect (p<.01).

^h Period effect (p<.10).

ⁱ Period effect (p<.05).

^j Period effect (p<.01).

^k Treatment * period interaction (p<.05).

density) was a reliable criterion for assessing mineral status of bone.

Calcium (% of ash) was not influenced by dietary P level ($p > .10$) or sampling period ($p > .10$), whereas Ca ($\text{mg} \cdot \text{cm}^{-3}$) was greater ($p < .10$) in HP than LP heifers and increased ($p < .01$) from the first to the second collection (Table 16). Rib P content (% of ash) was greater ($p < .05$) in HP heifers, but no period effects were observed for this parameter ($p > .10$). When expressed as $\text{mg P} \cdot \text{cm}^{-3}$, HP heifers had greater ($p < .01$) P content in rib cortical bone than LP heifers. Also P content ($\text{mg} \cdot \text{cm}^{-3}$) increased ($p < .01$) from the first to the second collection (Table 16). Magnesium, regardless of method of expressing its content in rib bone (either percent of ash or $\text{mg} \cdot \text{cm}^{-3}$) was affected by dietary P level and sampling period (Table 16).

When expressed on an ash weight basis, Ca, P and Mg did not show similar trends with respect to each other as they did when expressed on a per unit volume basis, that is when examined with respect to both dietary P level and sampling period, which would be expected due to the interrelationship of these minerals within bone and by the process through which bone is calcified or resorbed (Jacobson et al., 1972).

Little (1972b) found that the expression of P content per unit volume of fresh bone, following determination of specific gravity, was more sensitive than the expression per unit weight of dry, fat-free bone. Results obtained in the present experiment are in agreement with Little (1972b).

Similar Ca, P and Mg rib bone concentrations ($\text{mg}\cdot\text{cm}^{-3}$) to those in the present study have been reported by several researchers (Little, 1980; Hoey et al., 1982; Read et al., 1986b). Rib P values observed in LP heifers ranged from 88.9 to 176.4 $\text{mg}\cdot\text{cm}^{-3}$ and from 132.7 to 192.4 $\text{mg}\cdot\text{cm}^{-3}$ in HP heifers. Little (1980) reported an average rib bone P concentration of 144.1 $\text{mg}\cdot\text{cm}^{-3}$ in weanling cattle fed .12% P (DMB) for 10 wk, a P level identical to that fed the LP heifers.

Little and Shaw (1979) in a study of rib bone P in grazing cattle concluded that P levels in the 12th rib around 120 $\text{mg}\cdot\text{cm}^{-3}$ indicate P deficiency while levels over 150 $\text{mg}\cdot\text{cm}^{-3}$ indicate adequacy. These authors stress, however, that if rib bone P concentration ($\text{mg}\cdot\text{cm}^{-3}$) is used as a diagnostic tool, the nutritional history of the animals must be taken into account as it is possible for P concentrations higher than 150 $\text{mg}\cdot\text{cm}^{-3}$ to be observed in bone samples from P deficient animals. However, even though both groups would be considered "adequate" at the second collection, HP heifers maintained a clear advantage in rib P concentration (average difference of 17.2 $\text{mg}\cdot\text{cm}^{-3}$) (as well as for Ca and Mg).

It is interesting to note that ash content (%) indicated a difference between treatments in the first but not second collection, whereas, it is clear from rib mineral concentrations ($\text{mg}\cdot\text{cm}^{-3}$) that HP heifers had a greater amount of mineral present during both collections. This would indicate that percent ash may not be a sensitive indicator of bone mineral status, in agreement with the findings of Duncan (1958).

Breaking load (BL, kg) of 12th ribs was greater ($p < .05$) in HP than LP heifers (153.8 vs 114.3, respectively) (Table 17). Teh et al. (1982) reported greater breaking strength of rib (actually breaking load) in calves fed .31 versus .24%P for 56 d. Although BL values were greater in the present trial than those reported by Teh et al. (1982), trends were similar with higher P levels associated with increased BL. The consequences of increased breaking load is indicative of a greater amount of bone mineral in the ribs of HP heifers, in agreement with data collected by in vivo sampling. Also, during both bone biopsy collections, it was observed that ribs of LP heifers seemed "softer" than those of HP heifers. This observation stemmed from the fact that it required more pressure (i.e., force) on the drill and trephine to obtain samples from HP heifers. Breaking load is discussed in more detail in reference to mechanical properties of McIII in this chapter.

Based on results observed in this study, chemical and physical properties of rib bone are influenced by dietary P level. It would appear in this trial that .12% P (DMB) was marginal with respect to rib mineralization during the period of rapid growth. However, as the animals matured and growth slowed, more mineral was deposited in the rib, but levels still did not compare with those of heifers receiving .20% P (DMB).

Rib bone density ($\text{g}\cdot\text{cm}^{-3}$) and rib bone mineral concentration ($\text{mg}\cdot\text{cm}^{-3}$) seem to be most sensitive of rib parameters studied in relation to indicating bone mineral status, in agreement with the

Table 17. Influence of dietary phosphorus level on the breaking load (kg) of the 12th rib.

Item	Dietary Phosphorus Level ^{a, b}		
	LP	HP	SEM
Breaking Load (kg) ^c	114.3	153.8	11.9

^a LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20% P, DMB).

^b Mean $\pm$ standard error of the mean (SEM) based on 7 animals per treatment group (14 total); all ribs were collected at slaughter.

^c LP and HP means differ ($p < .05$).

findings of Little (1972b), Little and Minson (1977), and Judkins et al. (1985). However, in regard to these parameters and P status, Read et al. (1986b) point out that specific gravity (closely related to density) may be less sensitive than P content ($\text{mg}\cdot\text{cm}^{-3}$) in bone in subclinical aphosphorosis, but in more severe cases, equally conclusive in identifying P deficiency.

Vertebrae

The affects of dietary P level on various parameters associated with coccygeal vertebrae V, VI and VII collected at slaughter are shown in Table 18. All parameters examined exhibited similar trends in the three vertebrae with the exception of VBMC. Vertebral bodies progressively nearer the end of the tail (i.e., vertebra V is proximal to tail head) are smaller, therefore, containing a smaller absolute amount of bone mineral.

Percentage of bone ash was greater ($p<.10$) in HP than LP animals (63.8 v 62.8%, respectively) in vertebra VII. However, no differences between treatment groups were found in vertebra V and VI, although HP animals had a numerically greater percentage of ash than LP animals (Table 18). When expressed on an ash basis, P, Ca and Mg yielded similar results to that of percent bone ash in the vertebrae studied. Phosphorus and Mg (% bone ash) content of the three coccygeal vertebrae were unaffected by dietary P ($p>.10$). Calcium content (% bone ash) was greater in vertebra V ($p<.05$) and VII ($p<.10$) (Table 18) in HP heifers. No difference ($p>.10$) between treatment groups was observed for this parameter in vertebra VI

Table 18. Influence of dietary phosphorus level on various coccygeal vertebrae bone parameters.

Item ^a	Coccygeal Vertebra								
	Fifth			Sixth			Seventh		
	LP ^b	HP ^b	SEM ^c	LP	HP	SEM	LP	HP	SEM
Ash (%)	62.8	63.8	.39	63.4	63.8	.28	62.8 ^e	63.8 ^f	.37
Ca (%), ash	38.1 ^g	37.1 ^h	.30	38.2	37.2	.44	38.1 ^e	36.8 ^f	.45
P (%), ash	14.7	15.3	.30	14.9	15.4	.25	15.3	15.5	.23
Mg (%), ash	.57	.59	.02	.57	.59	.01	.56	.58	.02
VBMC (g) ^d	4.58 ^g	5.64 ^h	.30	3.79 ⁱ	4.72 ^j	.23	3.28 ^g	4.23 ^h	.25
Bone density (g·cm ⁻³)	1.21 ⁱ	1.39 ^j	.01	1.21 ⁱ	1.39 ^j	.02	1.21 ⁱ	1.39 ^j	.02
Ca (mg·cm ⁻³), fresh	289.0 ⁱ	330.0 ^j	3.1	292.5 ⁱ	330.6 ^j	5.4	290.3 ⁱ	327.3 ^j	1.9
P (mg·cm ⁻³), fresh	115.0 ⁱ	136.4 ^j	2.2	114.4 ⁱ	137.3 ^j	2.6	116.3 ⁱ	138.1 ^j	3.9
Mg (mg·cm ⁻³), fresh	4.36 ⁱ	5.23 ^j	.16	4.32 ⁱ	5.28 ^j	.16	4.25 ⁱ	5.13 ^j	.14

^a Means based on 7 animals per treatment group; all vertebrae collected at slaughter.

^b LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20%, DMB).

^c Standard error of the mean.

^d Vertebra bone mineral content.

^{e,f} Means within a vertebra row differ (p<.10).

^{g,h} Means within a vertebra row differ (p<.05).

^{i,j} Means within a vertebra row differ (p<.01).

although numerically greater in HP heifers. Duncan (1958) has pointed out that percentage bone ash gives no indication of status of mineral reserve and suggested that comparison of the ash weight of representative whole bones would be a better indication of mineral reserve and status. An example of this is bone resorption which involves the removal of both bone matrix and mineral matter, resulting in reduced bone mass and ash, but not necessarily ash percentage.

In agreement with the hypothesis of Duncan (1958), the VBMC (g), which represents the total amount of mineral present in each whole vertebra, gave an indication of the mineral status--with lower values ($p < .05$) for the LP group. The values represent a 23, 25 and 29% greater amount of bone mineral present in HP versus LP animals in coccygeal vertebra V, VI and VII, respectively. The low (.12%, DMB) P level in this study was not adequate for complete mineralization and/or caused mobilization of mineral from the vertebrae in response to low dietary P.

Bone density ($\text{g} \cdot \text{cm}^{-3}$) of fresh vertebrae was also sensitive to dietary P level, with HP heifers having greater ($p < .01$) vertebral density (1.39 vs 1.21, respectively, for all three vertebrae studied, Table 18). As discussed with ribs, reduced bone density is indicative of increased porosity in bone (i.e., reduced mineral content). This decrease may also be accompanied by an increase in water and/or fat within the bone. Little (1972b) has reported findings similar to these in ribs from cattle fed low P diets. Results of bone density of vertebrae are similar to those found in

rib biopsies in this study. This would suggest (as expected based on work by Benzie et al., 1959) that these bones acted in a similar manner in response to different dietary P level. This parameter also clearly ranked the treatment groups with respect to P level in this long term study.

When P, Ca and Mg were expressed on a per unit volume basis ($\text{mg}\cdot\text{cm}^{-3}$, fresh basis) differences between treatment groups ($p < .01$) were seen in all vertebrae studied with HP heifers having the greatest mineral concentrations (Table 18). This result would be expected based upon the observed VBMC and bone density data as mineral concentration per unit volume of bone takes into account both density and ash percentage and is in agreement with Little (1972b).

In the present study, VBMC, bone density and expressing mineral concentrations on a per unit volume basis were all sensitive to dietary P level in vertebrae and clearly ranked the two dietary P levels, whereas percent bone ash or mineral concentration expressed on an ash basis did not. These results are consistent with those observed in rib biopsy samples in this trial. Perhaps the tail biopsy technique described by Wilson and Hatfield (1974) would be a useful technique when assessing P status of cattle, not only from the aspect that vertebrae are quite sensitive to different P levels (as indicated in this study), but also in that the value of the carcass is not diminished as is a potential problem with the rib biopsy technique.

Third Metacarpals

Several properties of whole McIII were estimated using dual photon absorptiometry (DPA). The use of DPA has been described in detail elsewhere (Chapter Six). Results of DPA are shown in Table 19. The HP heifers had greater ($p < .01$) whole McIII bone mineral density (WBMD) and whole McIII bone mineral content (WBMC) than LP heifers, 1.61 vs $1.44 \text{ g} \cdot \text{cm}^{-2}$ and 129.8 vs 114.3 g , respectively. The WBMD estimate is a linear density as photon absorption provides no information at all about the depth dimension of bone. However, it is doubtful that a volume density is desired due to structure of McIII (e.g., irregularity of trabecular structure, bone marrow and variation due to the irregular pattern of haversian canals).

The differences detected in WBMD and WBMC from DPA scans in this experiment may point out the insidious nature of long-term marginal or low P intake. Figure 8 represents typical computer output generated by DPA scans performed on the McIII from the two treatment groups. The McIII from the HP treatment group (b) shows little bone mineral loss in either its proximal or distal ends indicated by the dark areas (i.e., trabecular bone), whereas both proximal and distal ends indicate loss of labile trabecular bone in the LP McIII (a). Also, bone mineral loss at the midshaft of LP McIII is evident, an area usually less labile, more stable, mature compact bone. Since the compact shafts of long bone are usually the last "mineral storehouse" affected in times of low intake (Benzie et al., 1959), it

Table 19. Influence of dietary phosphorus level on various whole third metacarpal (McIII) physical and chemical properties.

Item ^{b,c}	Dietary Phosphorus Level ^a		
	LP	HP	SEM ^d
WBMD ($\text{g}\cdot\text{cm}^{-2}$) ^h	1.44	1.61	.261
WBMC (g) ^g	114.3	129.8	3.09
CBI (%) ^f	45.0	48.6	.77
MLWT (mm) ^e	7.88	8.57	.187

^a LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20% P, DMB).

^b Means based on 14 measurements (7 right, 7 left McIII); 7 animals per treatment group.

^c WBMD = Whole McIII bone mineral density; WBMC = Whole McIII bone mineral content; CBI = Cortical bone index; MLWT = Medial lateral wall thickness.

^d Standard error of the mean.

^e LP and HP means differ ($p < .05$).

^f LP and HP means differ ($p < .01$).

^g LP and HP means differ ($p < .005$).

^h LP and HP means differ ($p < .001$).

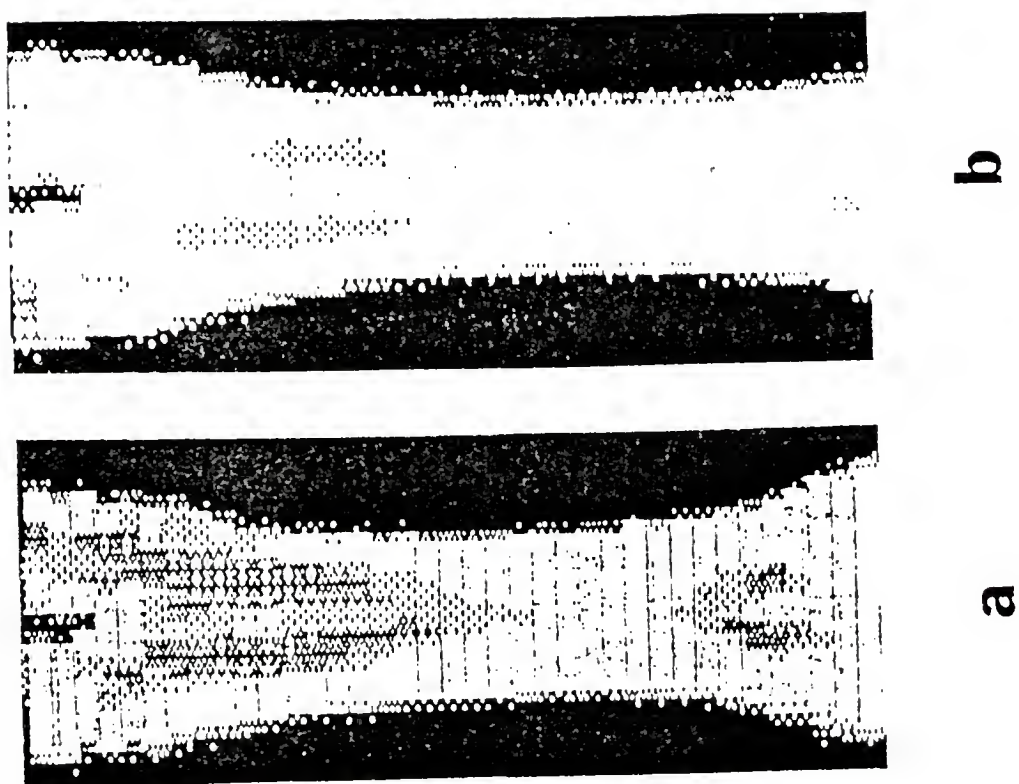


Figure 8. Typical dual photon absorptiometry (DPA) scans of third metacarpals from animals fed either .12% P (DMB) (a) or .20% P (DMB) (b).

is apparent from DPA scans that LP animals (which had been on the lower dietary P regimen for over 2 yr) were drawing upon mineral from these areas to help meet metabolic needs (this in addition to the lower mineral content observed in the rib and vertebra).

Although no physical signs of lameness or stiffness were observed in LP heifers in this study, one may assume that if these animals had been allowed to continue on this present regimen, including subsequent gestations and lactations that, in time, the marginal P level (.12% P, DMB) would most likely cause severe, if not debilitating, lameness, thus affecting the useful productive life of the animal. To this point, Call et al. (1982) reported that cows fed low dietary P (less than $7 \text{ g} \cdot \text{d}^{-1}$) for 14 to 24 mo prior to their eighth gestation showed varying signs of osteoporosis and other related bone changes, as well as some pathological and traumatic fractures.

Cortical bone index (CBI, %) estimated at the midpoint of McIII diaphysis using AP radiographs was greater ($p < .01$) in HP than LP heifers, 48.6 vs 45.0, respectively. Similarly, MLWT (mm) was also greater ($p < .05$) in HP than LP heifers, 8.57 vs 7.88, respectively. Both of these measurements corroborate the DPA scan analysis (i.e., there was less cortical bone at the midshaft of the McIII diaphysis of LP heifers). Similar findings were reported by Little (1984) of decreasing quantities of cortical bone measured at the midpoint of bone metatarsals in P deficient cows. He reported that the ratio of medullary width:total width was greatest in P stressed animals, while

the total widths were very similar between P stressed and supplemented cows.

The results of the mechanical tests performed on McIII along with various chemical properties and some geometrical measurements are shown in Table 20. Correlation coefficients between these various properties are shown in Table 21. Numerous types of tests are used to determine strength of materials; however, the flexure or bending test which is commonly used in examining the mechanical properties of bone was employed in this study. Currey (1970) reported that bending is a closer approximation to the kind of loading often causing failure in life than tensile loading, only if whole bone is used (as is the case in this study).

Frankel and Burnstein (1970) stated that the structure of bone responds to and is derived from its mechanical properties. Animal nutritionists have long used the mechanical properties of bone for determining the bioavailability of minerals and establishing nutrient requirements (Miller et al., 1962; Huagh et al., 1971; Schryver, 1978; Crenshaw et al., 1981b). Crenshaw et al. (1981a) concluded that a better understanding of principles involved in determining mechanical properties of bone would allow more accurate conclusions to be made concerning the effects of nutrients on mineralization, and more accurate comparisons to be made among various experiments.

This latter point must be addressed due to the anisotropic and viscoelastic nature of bone derived from the composite nature of bone material (i.e., the association between collagen and

Table 20. Influence of dietary phosphorus level on various mechanical and chemical properties and area indices of third metacarpals.

Item ^{b,c,d}	Dietary Phosphorus Level ^a		
	LP	HP	SEM ^e
Breaking Load ^g (kg)	1179	1348	53.2
Breaking Strength ^g (MPa)	189.2	202.5	3.55
Young's Modulus ^f (GPa)	2.5	2.8	.09
Strain	.076	.075	.0031
Moment of Inertia, Ellipse (cm ⁴)	2.43	2.59	.132
BMC (g·2cm ⁻¹) ^h	11.2	12.6	.33
Ash (%) ^h	67.2	68.0	.20
Ca (%), ash	37.2	37.1	.23
P (%), ash	16.3	16.4	.14
Mg (%), ash	.53	.54	.01
CAI (mm ²) ^h	932	1048	24.4
EAI (mm ²) ^g	660	729	15.2
XRI (mm ²) ^g	957	1069	29.9
PAI (mm ²) ^g	523	570	12.3

^a LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20% P, DMB).

^b Means based upon 14 samples per treatment group (7 right, 7 left third metacarpals).

^c BMC = Bone mineral content; CAI = Circular area index; EAI = Elliptical area index; XRI = Radiographic area index; PAI = Planimeter area index.

^d BMC, Ash, Ca, P, Mg, CAI, EAI, XRI and PAI were all measured from the 2 cm third metacarpal cross-section removed at the break point.

^e Standard error of the mean.

^f LP and HP means differ ($p < .10$).

^g LP and HP means differ ($p < .05$).

^h LP and HP means differ ($p < .01$).

Table 21. Correlation coefficients between various third metacarpal (McIII) diaphysis mechanical, chemical and physical properties (measured at point of failure).

Item ^{a,b}	BMC	Ash	P	Ca	Mg	BL	BS	E	Strain	MIE	CAI	EAI	XRI	PAI
BMC	-													
Ash	.728**	-												
P	.440 ⁺⁺	.687**	-											
Ca	.029	-.081	.169	-										
Mg	.143	-.120	-.188	.084	-									
BL	.884**	.516 ⁺⁺⁺	.397 ⁺⁺	.173	.223	-								
BS	.462 ⁺⁺	.368 ⁺	.290	-.010	.149	.538 ⁺⁺⁺	-							
E	.204	.445 ⁺⁺	.559 ⁺⁺⁺	.191	.012	.299	.604*	-						
Strain	.109	-.251	-.447 ⁺⁺	-.215	.061	.035	-.044	-.816**	-					
MIE	.684**	.309	.189	.152	.201	.806**	-.023	-.096	.130	-				
CAI	.753**	.562	.375 ⁺⁺	.171	.064	.712**	.305	.201	-.000	.604*	-			
EAI	.768**	.447 ⁺⁺	.271	-.008	.200	.783**	.198	.086	.061	.787**	.896**	-		
XRI	.741**	.323 ⁺⁺⁺	.324 ⁺	.134	.099	.750**	.454 ⁺⁺	.125	-.013	.559 ⁺⁺⁺	.902**	.824**	-	
PAI	.812**	.484 ⁺⁺	.344 ⁺⁺⁺	-.045	.215	.860**	.322 ⁺	.127	.111	.776**	.789**	.801**	.757**	-

a BMC = Bone mineral content; P = Phosphorus; Ca = Calcium; Mg = Magnesium; BL = Breaking load; BS = Breaking strength; E = Young's modulus; MIE = Moment of inertia-ellipse; CAI = Circular area index; EAI = Elliptical area index; XRI = Radiographic area index; PAI = Planimeter area index.

b All correlation coefficients based on 28 measurements.

+ (p<.10).

++ (p<.05).

+++ (p<.01).

* (p<.001).

** (p<.0001).

hydroxyapatite). Since bone is anisotropic, values will vary when tested across the grain in some cases by a factor of two or more. Viscoelastic properties signify that all results of mechanical testing will vary with the strain or loading rate, requiring that this parameter be specified. Increasing the loading rate reportedly increased the capacity for long bones to absorb energy (McElhaney, 1966). Bones may demonstrate nearly twice the stiffness at high speed loading rates compared to slow rates (Frost, 1973), thus as loading rate increases so will breaking strength.

The nominal full length of all McIII was approximately 18 cm. Force-deformation curves indicated that metacarpals from both groups showed little plastic behavior during the flexure test while undergoing loading prior to failure (i.e., they were more nearly elastic during testing). The BL (kg) was greater ($p < .05$) in HP than LP heifers, 1348 vs 1179, respectively. The BL represents the force at which any further increase in force results in failure of the bone. Breaking load is a structural test of bone and is determined by both the size and quality of the material (Lawrence, 1986). Crenshaw et al. (1981a) reported that total BMC is the most critical factor involved with BL. Table 20 shows that BMC (g) values recorded in the 2 cm McIII diaphysis cross-sections removed at the point of loading were greater ($p < .01$) in HP than LP heifers, 12.6 vs 11.2, respectively (again, one must also visualize the decrease in compact bone seen in DPA scans of LP heifers). The correlation coefficient between BL and BMC was $r = .884$ ($p < .0001$) (Table 21). This correlation

coefficient is similar to that reported by Lawrence (1986) of $r=.961$ for BL and BMC in a longitudinal study of McIII from horses 1 d to 33 yr of age. The relationship between BL and BMC is shown graphically in Figure 9 with the fitted linear regression model.

If normal bovine cortical bone is uniformly mineralized a linear relationship should exist between cortical area of McIII and BMC. The relationships between area indices and BMC are shown in Figures 10, 11, 12 and 13. All area indices appear to be good predictors of BMC with the more precise indices (i.e., PAI and EAI) having the highest correlations (Table 21) and the least scatter of the data. All cortical area estimates (i.e., CAI, XRI and EAI) were greater ($p<.05$) in the HP than LP heifers (Table 20) indicating bone mineral was distributed over a greater area in the HP animals as these estimates take into account both outside and inside diameters of the cross-sections. Area indices were highly correlated with BL (Table 21) as would be expected based on the relationships between BL and BMC as well as BMC and the area indices.

There are few reports in the literature that pertain to the mechanical properties of bovine McIII determined by flexure with respect to mineral nutrition, whereas, thorough investigations with equine (Schryver, 1978; Lawrence, 1986) and swine (Crenshaw et al., 1981b) have been reported. Becker and Neal (1930) reported data on bone "strength" in cattle fed diets low in Ca and P. Values reported as BS (but which actually represent BL) in McIII ranged from 710 kg in a Florida native cow receiving no supplemental Ca or P to

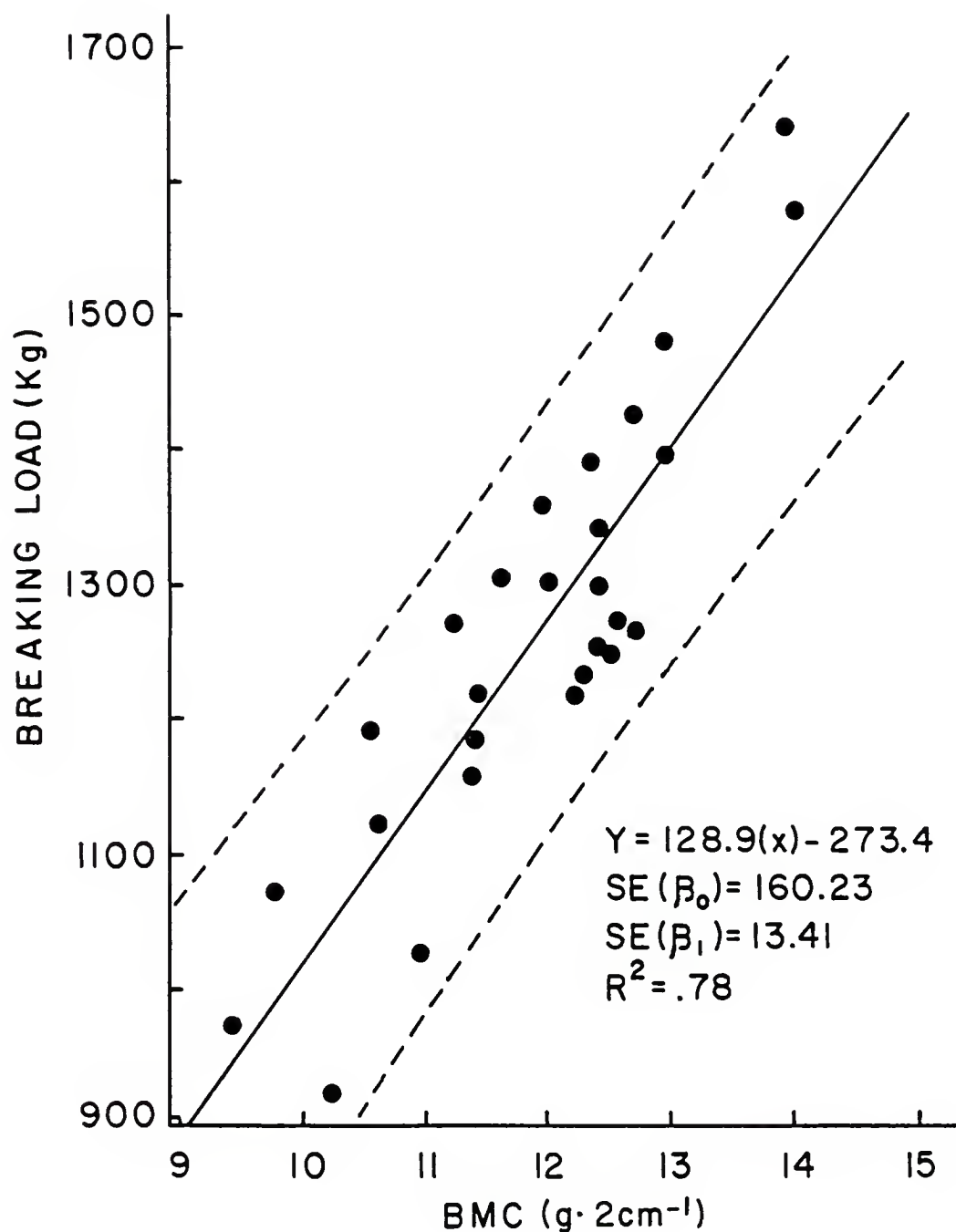


Figure 9. Relationship between breaking load (Y) and bone mineral content (BMC) (X) of third metacarpal break section. Dashed lines represent 95% confidence bounds. SE (β_0) = Standard error of intercept estimate; SE (β_1) = Standard error of slope estimate.

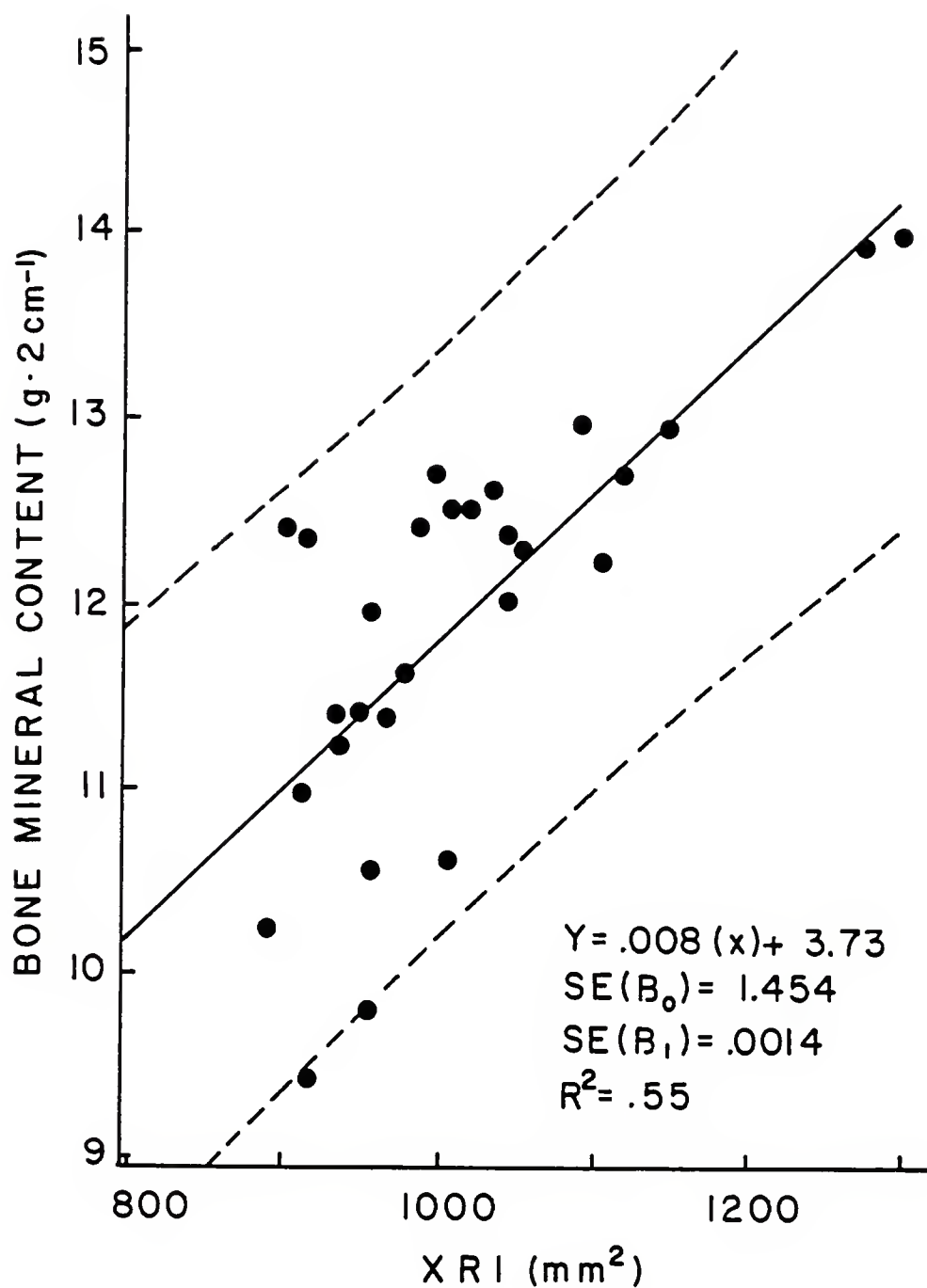


Figure 10. Relationship between bone mineral content (Y) and radiographic area index (XRI)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. SE (β_0) = Standard error of intercept estimate; SE (β_1)⁰ = Standard error of slope estimate.

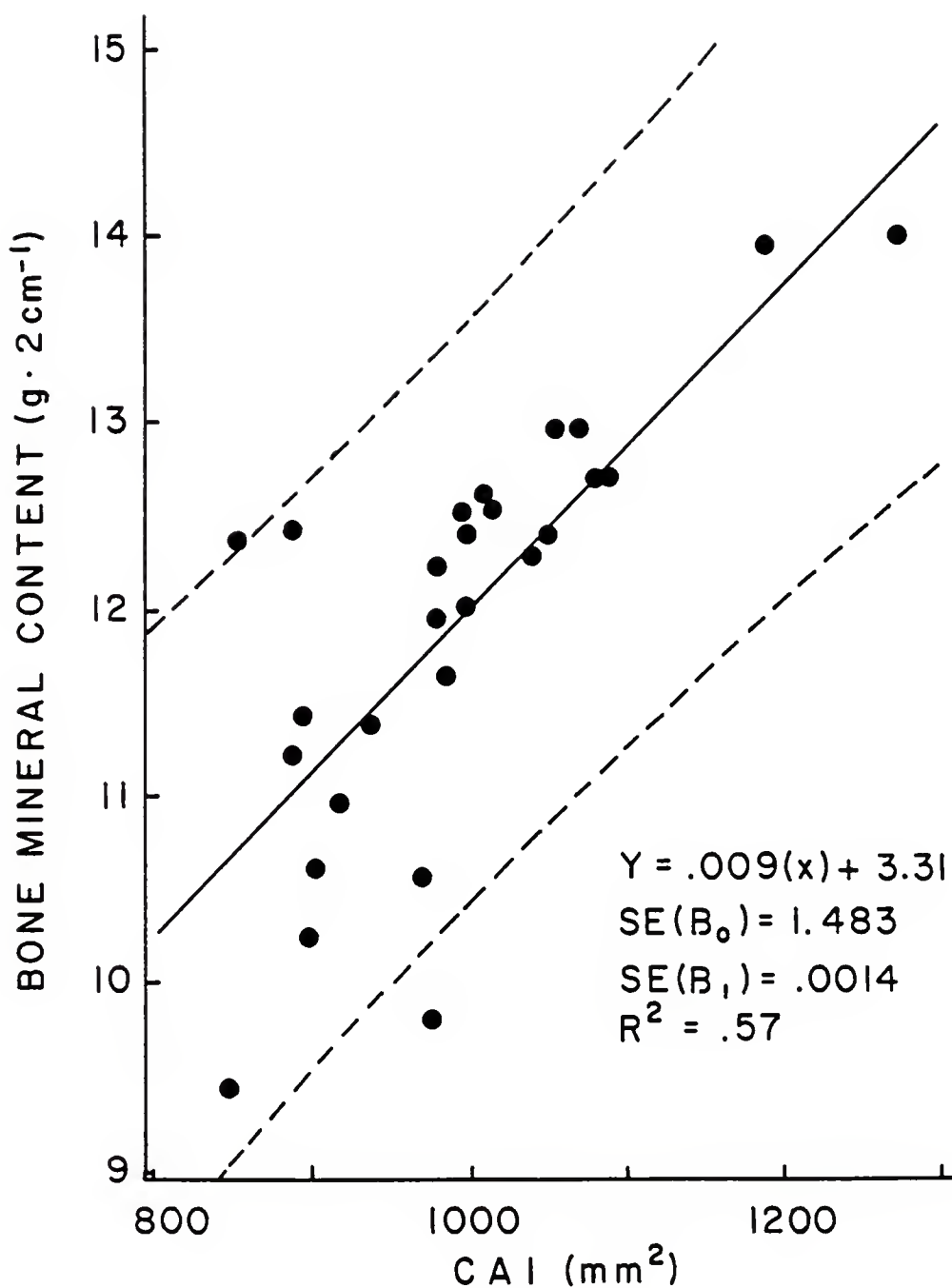


Figure 11. Relationship between bone mineral content (Y) and circular area index (CAI)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

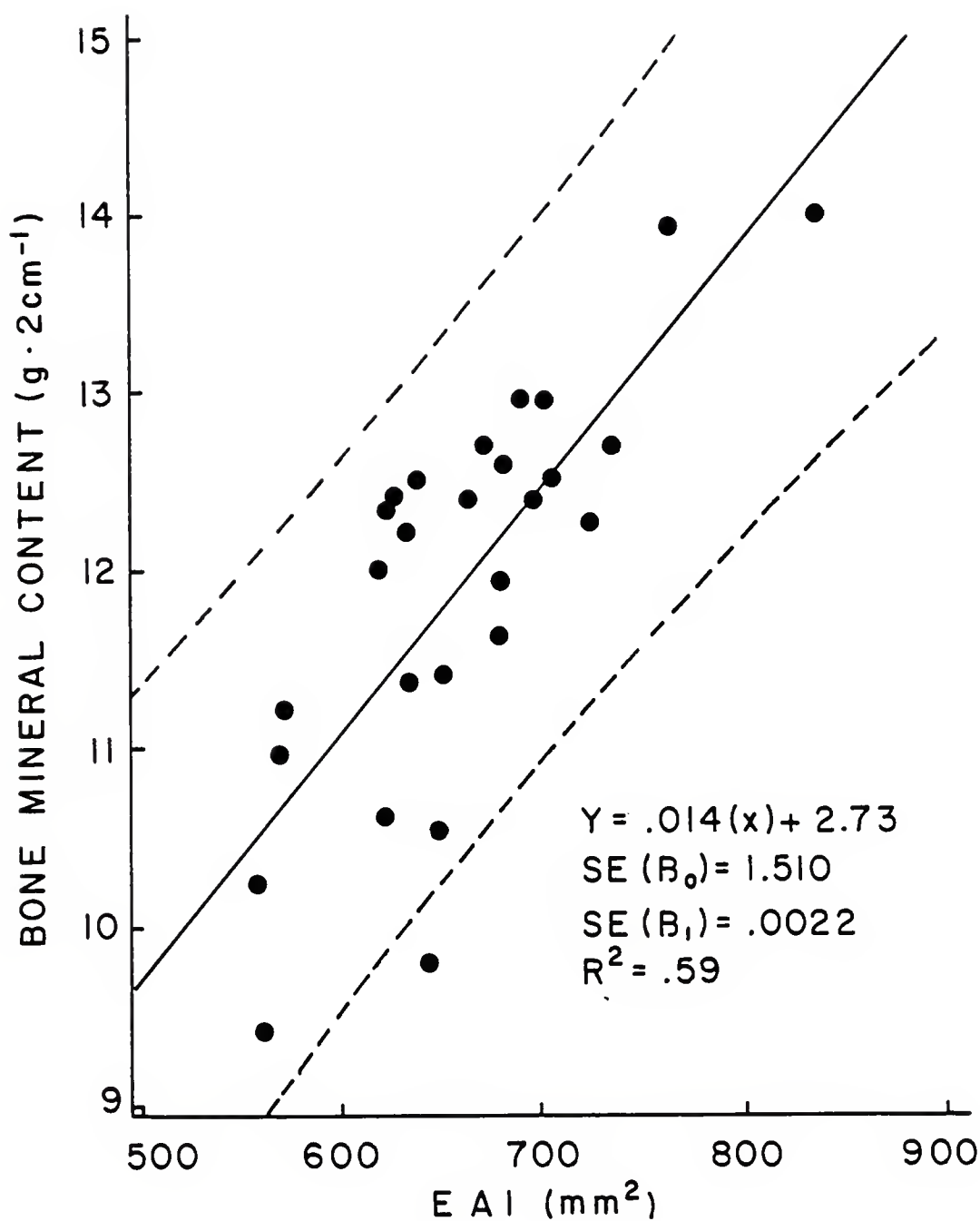


Figure 12. Relationship between bone mineral content (Y) and elliptical area index (EAI)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

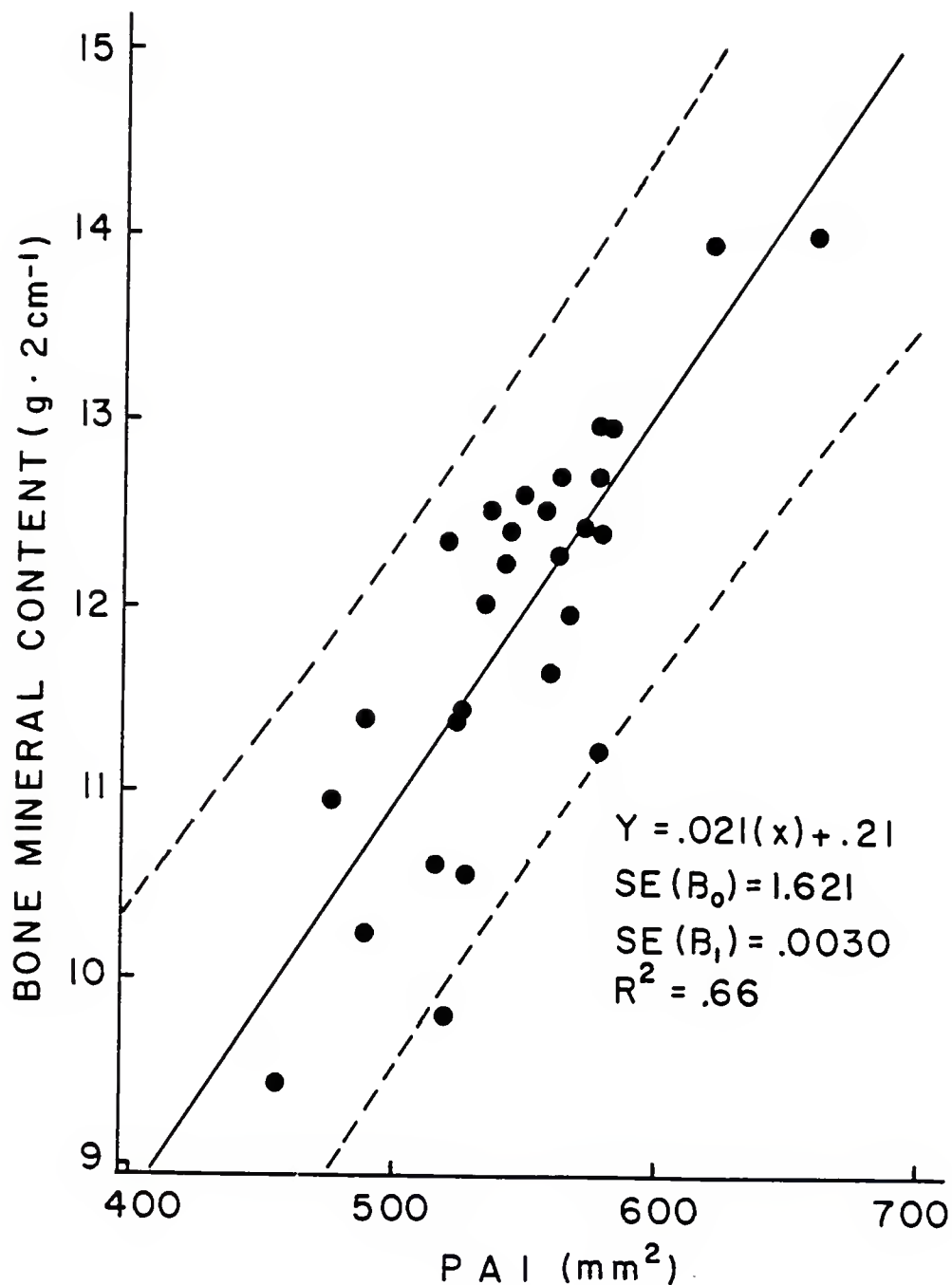


Figure 13. Relationship between bone mineral content (Y) and planimeter area index (PAI)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. SE (β_0) = Standard error of intercept estimate; SE (β_1)⁰ = Standard error of slope estimate.

1581 kg in a cow receiving a bonemeal supplement; however, no information was provided on loading rate. Breaking load values in the present study were in a similar range, that is, 920 to 1640 kg across both treatments.

Breaking strength (BS, MPa), or force per unit area of material, takes into account not only the cross-sectional area over which the force is applied but also the geometrical shape of this area, thus allowing comparison between bones that differ in both size and shape. This is accomplished by incorporation of moment of inertia (I) into the calculation of BS. Bone, being irregular in shape, makes calculation of I difficult; however, equations for calculating I from simple measures of known geometrical shapes have been derived. Lawrence (1986) reported that the use of a circle in estimating I in horses may result in a 100% overestimation of actual I, whereas, MIE closely approximated actual I. In the present study, McIII mid-diaphysis geometry was assumed to most closely approximate two ellipses. This assumption was based upon the observations that actual McIII cross-sectional area (PAI, mm^2) was most nearly approximated by EAI (mm^2) (Table 20) and was most highly correlated with EAI (Table 21); also cross-section geometry visually resembled an ellipse more so than a circle.

The estimated MIE (cm^4) of McIII from HP heifers was numerically greater than LP heifers (Table 20) indicating a larger distribution of material from the neutral axis of the McIII cross-sections. Although MIE was somewhat larger in HP McIII, greater ($p < .05$) BS (Table 20) was observed in McIII from this group, 202.5 vs 189.2 MPa,

respectively. This would indicate that, even though HP McIII had a greater area of bone material over which the load was distributed, this material was of higher quality. Crenshaw et al. (1981b) reported that increased stress is indicative of increased mineralization. This is in agreement with findings in this study as percent bone ash was greater ($p < .01$) in HP than LP heifers, 68.0 vs 67.2, respectively. However, no differences ($p > .50$) in Ca, P or Mg content expressed on an ash basis were observed between treatment groups (Table 20).

Magomedov (1978) has reported BS's of McIII in Black Pied heifers fed recommended P levels of 174 MPa; however, method of testing and loading rate were not reported. Recently, Lawrence (1986) using loading rates as in the present trial reported that BS of McIII reached a maximum of 211 MPa in horses between 1.5 and 4 yr of age (the same approximate age of heifers in this study), similar to BS values observed herein. As noted previously, studies pertaining specifically to mechanical properties of bovine bone as affected by dietary P level could not be found. However, other researchers have reported increases in bone strength due to an increase in the level of Ca and P fed in other species (Miller et al., 1962; Cromwell et al., 1972; Crenshaw et al., 1981b). Care, however, must be taken in comparing these results with those of the present study due to such factors as species difference, age at testing, Ca:P ratio, loading rate, length between roller supports, method of preparation and point of load application.

The relationship between BS and percent ash has been studied in several species. In a classic study, Vose and Kubala (1959) working with human femurs reported that BS increased rapidly with small increases in ash and fit an exponential curve to the data as they felt a linear curve would not make "biological" sense. Currey (1969) explained the lack of a linear relationship between BS and percent ash in failure of bone to completely mineralize as mineralization beyond an optimum value would decrease the resiliency of bone and reduce its ability to resist dynamic loading. This is an important concept as during the daily activities of an animal the bones of the skeleton, individually and collectively, are subjected repeatedly to a variety of force systems by gravity, muscular activity and various extrinsic factors. Consequently, the capacity of bone to withstand repetitive loading without breaking is of considerable importance with respect to fractures, especially the so-called fatigue of stress fractures.

The relationship between bone mineral and collagen in bone matrix has been suggested (Currey, 1969) to be responsible for the correlation of BS and percent ash. As percent ash values reach 63 to 68%, the organization of bone matrix produces optimum strength. Below this point it is thought that hydroxyapatite crystals disrupt collagen molecules when a load is applied, and above this range the apatite needles coalesce allowing cracks in the solid phase which then are not dissipated by the collagen matrix (Currey, 1969).

El Shorafa et al. (1979) reported a correlation of $r=.58$ between BS and percent ash in a longitudinal study of equine McIII. In the

present study, the correlation was $r=.368$ ($p<.10$) (Table 21). Although the correlation was somewhat lower, the narrow constraints of age and sex present in this study must be considered in terms of comparing results with more longitudinal studies. The relationship is shown graphically in Figure 14. The relationship between BS and BMC has also been investigated. Lawrence (1986) reported a correlation between these variables of $r=.643$, whereas in the present study, the correlation was $r=.462$ ($p<.05$) (Table 21). The relationship is shown graphically in Figure 15. It is interesting to note that this correlation (BS and BMC) is greater than percent ash and BS as it does not take account of collagens influence in strength of bone. Perhaps it is suggestive of the greater relationship between mineral content and compressive strength.

As bone develops, the inorganic mineral fraction is deposited within the protein matrix in a highly organized manner with a specific mineral to protein ratio. Optimum mineralization produces the lightest bone (contained in the smallest area) to give effective adequate support for locomotion (Lawrence, 1986). Optimum bone mineralization occurs when BS is maximized (Currey, 1969), below this level the mineral matrix is less organized. Increases in BS and BL up to the point of optimum bone mineralization reflects increased mineralization within the bone matrix and in the total amount of bone present. Above the point of optimum bone mineralization the organization of mineral within the matrix is not changed, but a greater amount of total bone is deposited. Miller et al. (1962)

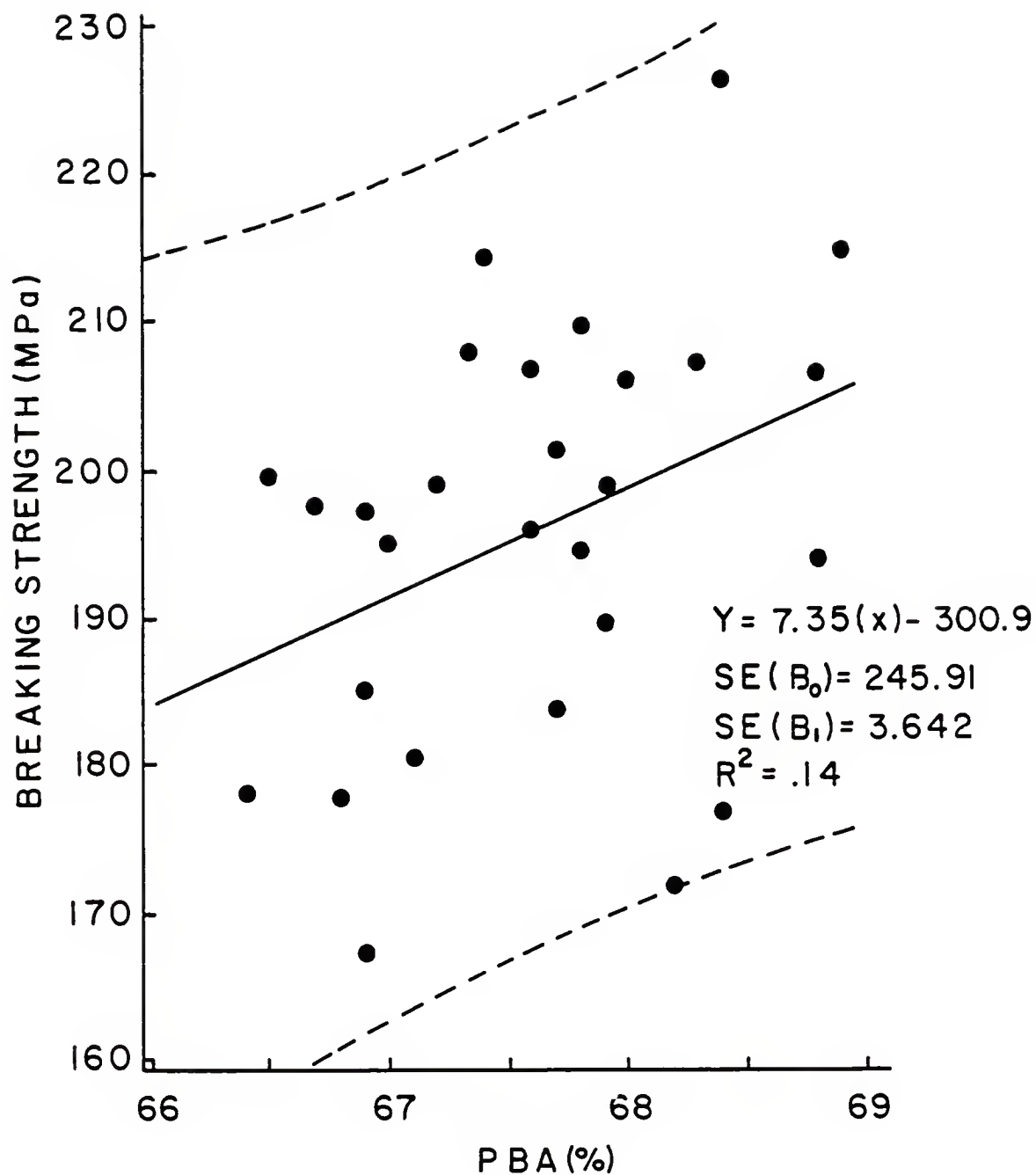


Figure 14. Relationship between breaking strength (Y) and percent bone ash (PBA)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. SE (β₀) = Standard error of intercept estimate; SE (β₁) = Standard error of slope estimate.

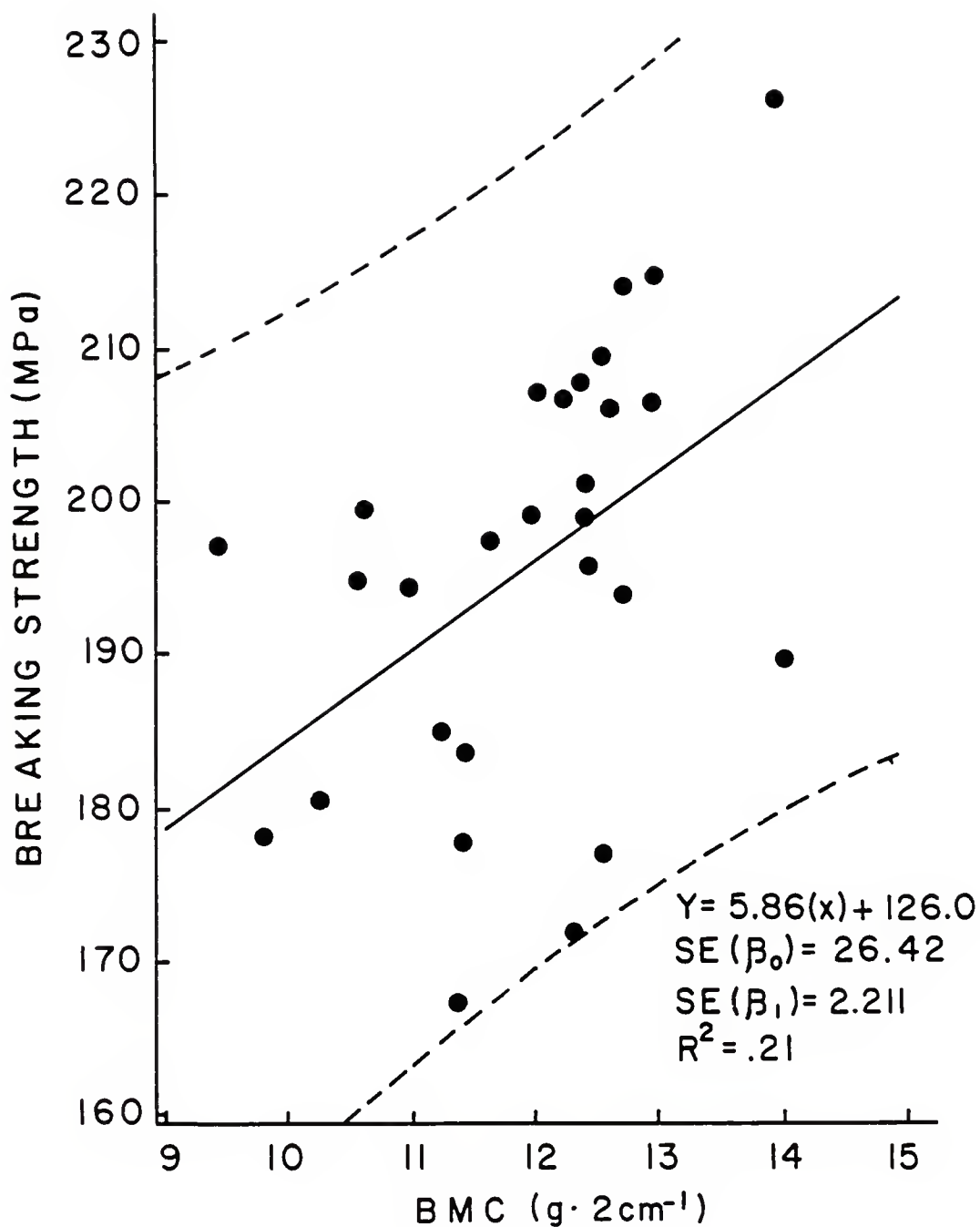


Figure 15. Relationship between breaking strength (Y) and bone mineral content (BMC)(X)--third metacarpal break section. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

and Crenshaw et al. (1981b) working with pigs have shown that BS reaches a maximum before BL which continues to increase as more total bone is deposited. Although optimum mineralization of bone matrix is reached at the highest stress, more total bone may be important to maintaining structural integrity of bone, especially in those animals expected to have long-term productive use. This may be desirable in establishing recommended versus minimum requirements.

The crux of the previous discussion is that mechanical characteristics of bone may yield different responses when influenced by various biological factors including dietary manipulation--and careful interpretation must be given to the results. If the load (force) required to cause failure in a given bone is less than another, no information is gathered as to whether the quality of the bone material is different, or whether the shape, amount and arrangement of the bone material is different. Thus it is possible that the load required to break a bone is greater than another but that it withstands less force per unit area of bone material.

Young's modulus (E) was greater ($p < .10$) in HP than LP heifers, 2.8 vs 2.5 GPa, respectively, suggestive of greater ratio of bone mineral to protein in the bone matrix (Table 20). This value is indicative of the stiffness of bone under normal stress. It (E) is a much more important mechanical characteristic of bone than is generally realized. If bone were considerably less stiff than it is, design of skeletons would be altered appreciably. For example, long bones would have to be made stouter to keep them from collapsing

sideways when loaded in compression. A stiffer material (as that seen in HP heifers) will require a greater stress for a certain strain or a greater load for a given deformation than will a more flexible material.

Currey (1969) has reported a linear relationship between E and percent ash and postulated that the resistance of the bone material to static stresses is related to percent ash, but not necessarily for dynamic stresses. Correlations between E and percent ash ($r=.445$, $p<.05$) and BMC ($r=.204$) (Table 21) in this study tend to agree with the hypothesis of Currey (1969). Strain (change in length per unit length) was not different ($p>.10$) between the treatment group (Table 20).

Results of BS, E and strain would suggest that HP McIII were also more resilient than LP McIII as recoverable strain energy would be greater, as would absorbed strain energy prior to rupture. Resilience of McIII would be an important physiological trait of these bones under field conditions as the chance of fracture would be diminished in the range of normal stress.

Conclusions

Results of this study indicate that the chemical and physical properties of bovine bones are sensitive to dietary P level. With respect to rib bone, it would appear that .12%P (DMB) was marginal with respect to rib mineralization compared to heifers receiving .20%P (DMB). It would also appear that as growth in the heifers tapered off more mineral was deposited in ribs of all heifers, as is

seen in increased bone ash and mineral concentrations in the ribs at the second biopsy collection (day 294 of the P supplementation phase). Of all parameters studied in rib bone, rib bone density ($\text{g}\cdot\text{cm}^{-3}$) and mineral content expressed on a per unit volume basis ($\text{mg}\cdot\text{cm}^{-3}$) seemed to be the most sensitive. Break load results of 12th ribs also indicated a greater amount of mineral present in heifers receiving .20%P (DMB).

Vertebral BMC, bone density and mineral concentrations on a per unit volume basis were also sensitive to dietary P level and clearly ranked the two dietary P levels, whereas percent bone ash or mineral concentration expressed on an ash basis did not. These results are consistent with those observed in rib biopsy samples in this trial.

Mechanical properties of bovine McIII were quite sensitive to dietary P level in this study. These properties clearly ranked the treatment groups as to dietary P level. Results indicate that McIII from HP heifers had both a greater quantity and quality of bone material. The lower dietary P level (.12%, DMB) utilized herein appears to be inadequate for optimum bone mineralization and strength of McIII based upon lower BS values with respect to the .20%P level. Also, HP heifers had a greater total mass of bone present in McIII based on BL and BMC values. These parameters may be of particular interest in regard to animals in the breeding herd which are expected to maintain a given level of production under ever changing field conditions for long periods of time.

Results of this study would suggest that the use of selected chemical and physical parameters of various bones would allow

reliable and objective assessment of P status in ruminants. The use of various bone properties as indicators of status or as diagnostic guides would necessitate in field use that the nutritional history of the animals in question be known.

It is also evident, based upon review of the literature, that more research is needed in the area of mechanical properties of bovine bone with respect to specific mineral projects as no significant data base presently exists.

CHAPTER SIX
NONINVASIVE TECHNIQUES TO ASSESS
PHOSPHORUS STATUS AND PREDICT BONE MINERAL CONTENT
AND BONE STRENGTH IN THE BOVINE

Introduction

Objective and reliable methods of assessing bovine phosphorus (P) reserves have been earnestly sought by animal scientists for many years. Since the skeleton is the major body reserve of P (Duckworth and Hill, 1953), it would appear to offer a useful sample area. Consequently, much work has been done to develop appropriate skeletal criteria for determining body P status from skeletal measurements.

Mineral status of an animal's skeleton can be simply determined by ash analysis of bone; however, this necessitates either surgery or slaughter to obtain a sample and may not be the preferred method of choice, especially for following bone development in the same animal over a period of time.

Several noninvasive methods of analyzing bone mineralization and density in vivo have been developed in the past 25 yr. Included in these are photon absorptiometry (PA) (Cameron and Sorenson, 1963), radiographic photometry (RP) (Ekman et al., 1970), neutron activation analyses (Al-Hiti et al., 1976), computerized axial tomography (Reich et al., 1976) and ultrasound (U) (Pratt, 1980). Such noninvasive methods, which provide estimates of bone mineral content (BMC), are both of interest and use to clinicians and scientists in evaluating

sequential changes in bone density and other bone parameters. The quantification of bone mineral kinetics is valuable in assessing skeletal changes that may be associated with such factors as normal skeletal development, nutritional influences, gerontologic demineralization, restricted physical activity, or endocrine and renal imbalancing conditions with secondary bone effects.

The more "commercially" available techniques of PA, RP and U have received considerable attention recently for evaluating bone density in both small and large animal studies. However, little work has been done in the bovine with any of these techniques. Also, these techniques are in various phases of development with respect to their ability to predict BMC and bone strength in all species.

The present project was undertaken to 1) study the effectiveness of PA, RP and U as techniques in delineating the quantity of bone mineral in third metacarpals (McIII) obtained from beef heifers fed different dietary levels of P, 2) evaluate the ability of these three techniques to predict BMC as determined by ashing and bone strength as determined by flexure test in the bovine and 3) provide an initial data base of simultaneous use of these three techniques on a common set of bovine McIII.

Materials and Methods

The duration of the present experiment ranged from 525 to 772 d. The experiment consisted of a P depletion phase (270 d), an adaptation period to the basal P supplementation diet (10 d) and a P supplementation phase (ranging from 245 to 492 d in length; common

experimental endpoint was day 245 of the P supplementation phase for nonpregnant and 3 wk postpartum for pregnant heifers).

Fifteen weaned Angus heifers, 7 to 8 mo of age, averaging 160 ± 3 kg initially, were housed under dry lot conditions on concrete floors in a covered barn and allowed ad libitum intake of a low P diet (Table 2) during the 270 d P depletion phase. At the end of this period heifers were allowed a 10 d adaptation period to the basal P supplementation diet (Table 2). In the P supplementation phase of the experiment, heifers (averaging 210 ± 6 kg) were allotted randomly ($7 \text{ animals} \cdot \text{group}^{-1}$) to one of two dietary P levels: 1) continuation of low P (LP) basal diet containing .12% P (dry matter basis, DMB) or 2) high P (HP) diet which consisted of basal diet supplemented to provide .20% total P (DMB) (Table 2). Basal diets fed during the course of the experiment were formulated to be low in P yet provide adequate energy, nitrogen, other minerals and vitamins to promote $.5 \text{ kg} \cdot \text{d}^{-1}$ gain. Calcium to P ratios in the P supplementation phase ranged from 3.1:1 in the HP diet to 5.2:1 in the LP diet (6:1 in P depletion phase).

Animals in this second phase of the experiment were group fed ad libitum their respective diets for the initial 210 d of the P supplementation phase. Feed intake was then restricted in an attempt to limit body condition in order to minimize potential calving difficulties.

All animals in both treatment groups were bred naturally via 120 d exposure to a single bull. Two heifers from each treatment were determined via rectal palpation as nonpregnant and were

subsequently slaughtered on day 245 of the P supplementation phase. Remaining pregnant heifers (5 per group) were maintained until 3 wk postpartum. Left and right McIII were collected from all heifers at slaughter. Calving occurred between day 386 and 471 of the P supplementation phase.

At collection, McIII were wrapped in .9% saline soaked gauze then frozen (-15 C) until further analysis. All soft tissues including fibrous periosteum were removed; care was taken not to scratch or disrupt the surface of the bones. While dissecting out all surrounding soft tissue, McIII were always kept immersed in .9% saline. Soft tissue free McIII were wrapped in .9% saline soaked gauze, placed in plastic bags and frozen (-15 C) prior to testing.

A Lunar Radiation Corporation¹ analyzer was used to estimate bone mineral content and bone mineral density by dual photon absorptiometry (DPA). This densitometer utilizes a highly collimated radionuclide source emitting a radiation beam at two separate energies, allowing DPA to account for soft tissue effects. The McIII during scanning were submerged in .9% saline which has an absorption pattern similar to soft tissue. The whole McIII was scanned and count information stored on a microcomputer floppy diskette. The scan line, which is 4.5 mm, allows sections of particular interest to be examined at a later time. Five scan lines (2.25 cm) were read at both the midpoint of the McIII diaphysis and a point 3 cm proximal to

¹ Lunar Radiation Corporation, Bone Mineral Analyzer Dual Photon Rectilinear Scanner Model DP3, Madison, WI.

the midpoint (approximately 1.125 cm on either side of these points). Photon absorption bone mineral content ($\text{g} \cdot 2.25 \text{ cm}^{-1}$) and bone mineral density ($\text{g} \cdot \text{cm}^{-2}$) were determined for these scan sections.

Anteroposterior (AP) radiographs were taken of all McIII with an aluminum stepwedge¹ simultaneously exposed as a reference standard. Radiographs were obtained using mobile x-ray equipment² and veterinary x-ray film³ in cassettes with compatible intensifying screens. Voltage, amperage, exposure time, film focal distance, film developing and processing were performed as described by Meakim et al. (1981). Photometric scans of the stepwedge and bone (at the midpoint and 3 cm proximal to the midpoint) were performed using a densitometer⁴ coupled with a microcomputer⁵ and digitizer.⁶ This provided an estimate of bone mineral content in radiographic bone aluminum equivalents (RBAE, mm Al).

High frequency ultrasound was used to evaluate BMC and bone strength by measuring elapsed time and distance over which a standardized pulse of sound transmitted mediolaterally through McII

¹ Atomic Products Corp., Atomic Lab Div. Center, Al Stepwedge Penetrometer, Moriches, NY.

² Universal X-Ray Products, Easymatic Super 325, Chicago, IL.

³ 3M Corp., Standard Veterinary X-Ray Film No. 1414, St. Paul, MN.

⁴ Southern Micro Instruments Inc., MicroComp Digital Image Analysis System, Atlanta, GA.

⁵ Zenith Data System Corp., Model Z-150 PC, St. Joseph, MI.

⁶ Houston Instrument, HiPad Digitizer, Austin, TX.

traveled (Figure 3). Ultrasound measurements were performed at the same points evaluated previously by DPA and RP. An ultrasonic generator¹ transmitted and received signals via matched KB Aerotech 1 MHz transducers mounted on an electronic digital caliper.² Liberal quantities of coupling medium were used to ensure good sound transmission. It was only possible to obtain consistent transmission of sound over the region of the McIII shaft that had reasonably parallel sides. The elapsed time between transmission and reception of a signal (t , μs) was displayed on an oscilloscope.³ The mediolateral diameter of McIII (d , mm) was measured simultaneously by the electronic caliper. The "effective" ultrasonic velocity (U , $\text{m}\cdot\text{s}^{-1}$) of sound pulses through McIII was then calculated using the formula $U = d/t$.

After all noninvasive tests were complete mechanical properties of whole wet McIII were determined by three-point bending using an Instron Floor Model Testing Machine⁴ at a deformation rate of $50 \text{ mm}\cdot\text{min}^{-1}$ as previously described in Chapter Five. Breaking load (BL), breaking strength (BS), Young's modulus (E), strain and moment of inertia of an ellipse (MIE) were determined as previously described (Chapter Five) for McIII. Broken McIII were reassembled and placed in plastic containers slightly larger than all McIII

¹ Panametrics, Pulser Receiver 5055 PR, Waltham, MA.

² Fowler, Ultracal Electronic Calipers, Detroit, MI.

³ Ballanline Lab Incorporated, Model 1023 AB, Boonton, NJ.

⁴ Instron Corporation, Model 1125, Canton, MA.

dimensions and liquid urethane¹ poured around them. Once solidified, the liquid urethane provided a flat, uniform surface enabling accurate removal of McIII diaphysis cross-sections using a table band saw. Two 2 cm McIII diaphysis cross-sections were removed: one at the midpoint (i.e., the break point) and the other 3 cm proximal to the midpoint (1 cm on either side of these points). These 2 cm sections corresponded to those points evaluated by RP, DPA and U.

Bone cross-sections were photographed along with a reference standard and actual area (PAI, mm²) irregardless of geometry determined by planimeter.² Caliper cross-section measurements from bone and radiographs were determined for AP bone diameter (B), AP medullary diameter (b), lateromedial (LM) bone diameter (D) and LM medullary diameter (d) (Figure 4). Equations for calculating McIII cross-section area indices (mm²) were $(B^2 - b^2)$ for circular area index (CAI) from bone and radiographic area index (XRI) from AP radiographs and $(BD - bd)$ for elliptical area index (EAI) from bone.

In an attempt to better approximate the "path" sound takes as it travels through the bone, ultrasound velocity was determined using distance traveled as the McIII cross-sectional area index (i.e., PAI, CAI, XRI and EAI). Planimeter ultrasound (PLAN-US), elliptical ultrasound (ELL-US), circular ultrasound (CIR-US) and radiographic ultrasound (XRAY-US) velocity were calculated and expressed as m²·s⁻¹.

¹ Bradson Auto Marine, Incorporated, Brad Foam, Pinellas Park, FL.

² Numonics Corporation, Model 1250-1, Lansdale, PA.

The McIII sections were dried for 12 h at 105 C, ether extracted in a soxhlet until no lipid material was observed in the ether (minimum 48 h) and ashed at 600 C for 12 h. Bone ash was expressed as $\text{g} \cdot 2 \text{ cm}^{-1}$ section and defined as BMC.

Paired t-tests (Montgomery, 1976) were performed to determine if the noninvasive measurements, BMC and mechanical properties measured in right and left McIII were similar in pairs of bones. After it was determined that parameter responses were similar for bones within an animal, average side response was computed and used in subsequent statistical analysis.

All data were analyzed by analysis of variance using the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS, 1982). Correlation coefficients between various parameters were estimated using the PROC CORR procedure of SAS (1982). Graphical representation of relationships between various parameters were generated using the PROC GPLOT procedure of SAS (1981); related linear regression equations were determined using the PROC REG procedure of SAS (1982).

Results and Discussion

Table 22 shows results of using noninvasive techniques on McIII to distinguish between two groups of beef heifers fed different dietary levels of P. Also included in this table are BMC values determined by ashing McIII diaphysis sections. Third metacarpals were chosen for studying these techniques due to their accessibility,

Table 22. Influence of dietary phosphorus level on bone mineral content (BMC), photon absorptiometry, radiographic photometry and ultrasound measurements at two locations along the bovine third metacarpal (McIII) diaphysis.

Item ^b	McIII Location ^a				
	Midpoint			3 cm Proximal to Mid Point	
	LP ^c	HP ^c	SEM ^d	LP	HP
BMC ($\text{g} \cdot 2 \text{ cm}^{-1}$)	11.2 ^g	12.6 ^h	.33	13.0 ^g	14.7 ^h
Photon Absorption-Bone Mineral Content ($\text{g} \cdot 2.25 \text{ cm}^{-1}$)	12.1 ^g	13.9 ^h	.39	13.9 ^e	15.4 ^f
Photon Absorption-Bone Mineral Density ($\text{g} \cdot \text{cm}^{-2}$)	1.39 ^g	1.55 ^h	.025	1.64 ^e	1.79 ^f
Radiographic Photometry (RBAE; mm, Al)	13.6 ^g	15.0 ^h	.25	15.1 ^g	16.9 ^h
Ultrasound ($\text{m} \cdot \text{s}^{-1}$)	3252 ^e	3354 ^f	34.3	3302 ^e	3406 ^f

^a McIII locations were at the midpoint and 3 cm proximal to the midpoint of the diaphysis. All measurements were made prior to mechanical testing.

^b Means based on 14 measurements per treatment group (7 right, 7 left McIII); 7 animals per treatment group.

^c LP (low phosphorus; .12% P, DMB); HP (high phosphorus; .20% P, DMB).

^d Standard error of the mean.

^{e,f} Means within a McIII location row differ ($p < .05$).

^{g,h} Means within a McII location row differ ($p < .01$).

susceptibility to mechanical stress and because it is one of the most active bones in growing cattle (Landau et al., 1985).

Bone mineral content ($\text{g} \cdot 2 \text{ cm}^{-1}$) was greater ($p < .01$) in both 2 cm sections removed from McIII diaphysis of HP heifers (Table 22). The correlation between BMC and BS ($r = .462$; $p < .05$) was reported previously in Chapter Five (Table 21). Breaking strength represents the force withstood by bone and the distribution of the area over which the force was applied. The decreased BS values observed in LP heifers are indicative of decreased mineralization of McIII osteoid material--seen as lower percentage ash and BMC (Chapter Five). Breaking load, the ultimate force required to break a bone, was highly correlated ($r = .884$; $p < .0001$) with BMC (Chapter Five; Table 21). Breaking load is dependent on both size and quality of osseous material, with total BMC the most critical factor (Crenshaw et al., 1981a).

Figures 16, 17, 18 and 19 represent the relationships between McIII cross-sectioned area indices and BMC. If normal bovine cortical bone is uniformly mineralized, a linear relationship should exist between cortical area and BMC, as can be seen from the graphs. All cross-sectional area indices were good predictors of BMC with the highest correlation coefficients observed for EAI ($r = .312$; $p < .0001$) and PAI ($r = .842$; $p < .0001$) (Table 23). Less scatter of data can be seen as the estimate of cortical area becomes more accurate. These results would indicate that medullary contents do influence the BMC as postulated by other workers (Meema et al., 1964; Anderson et al., 1956) and suggest that small refinements in area estimates

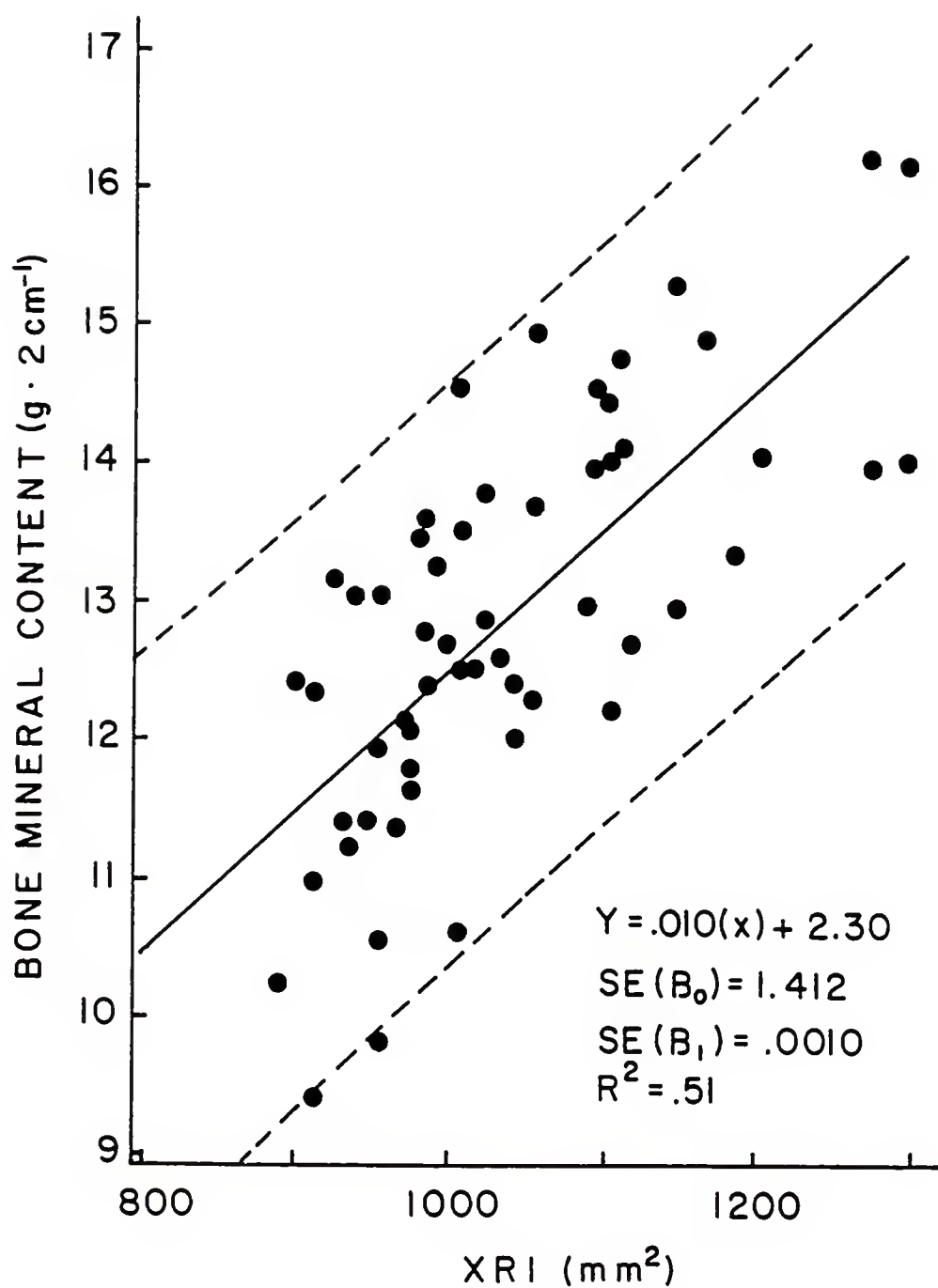


Figure 16. Relationship between bone mineral content (Y) and radiographic area index (XRI) (X) from two 2 cm third metacarpal cross-sections. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

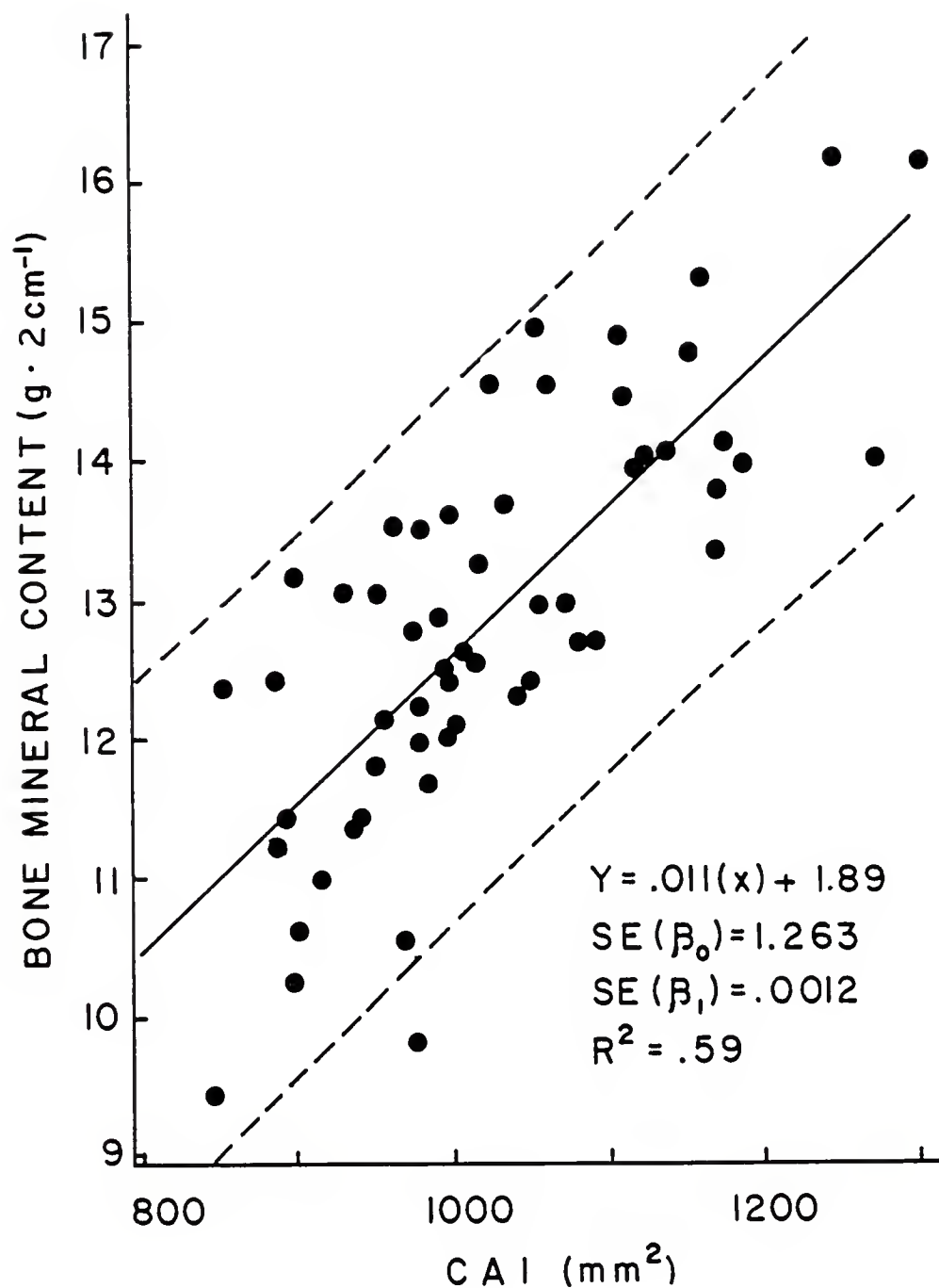


Figure 17. Relationship between bone mineral content (Y) and circular area index (CAI) (X) from two 2 cm third metacarpal cross-sections. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

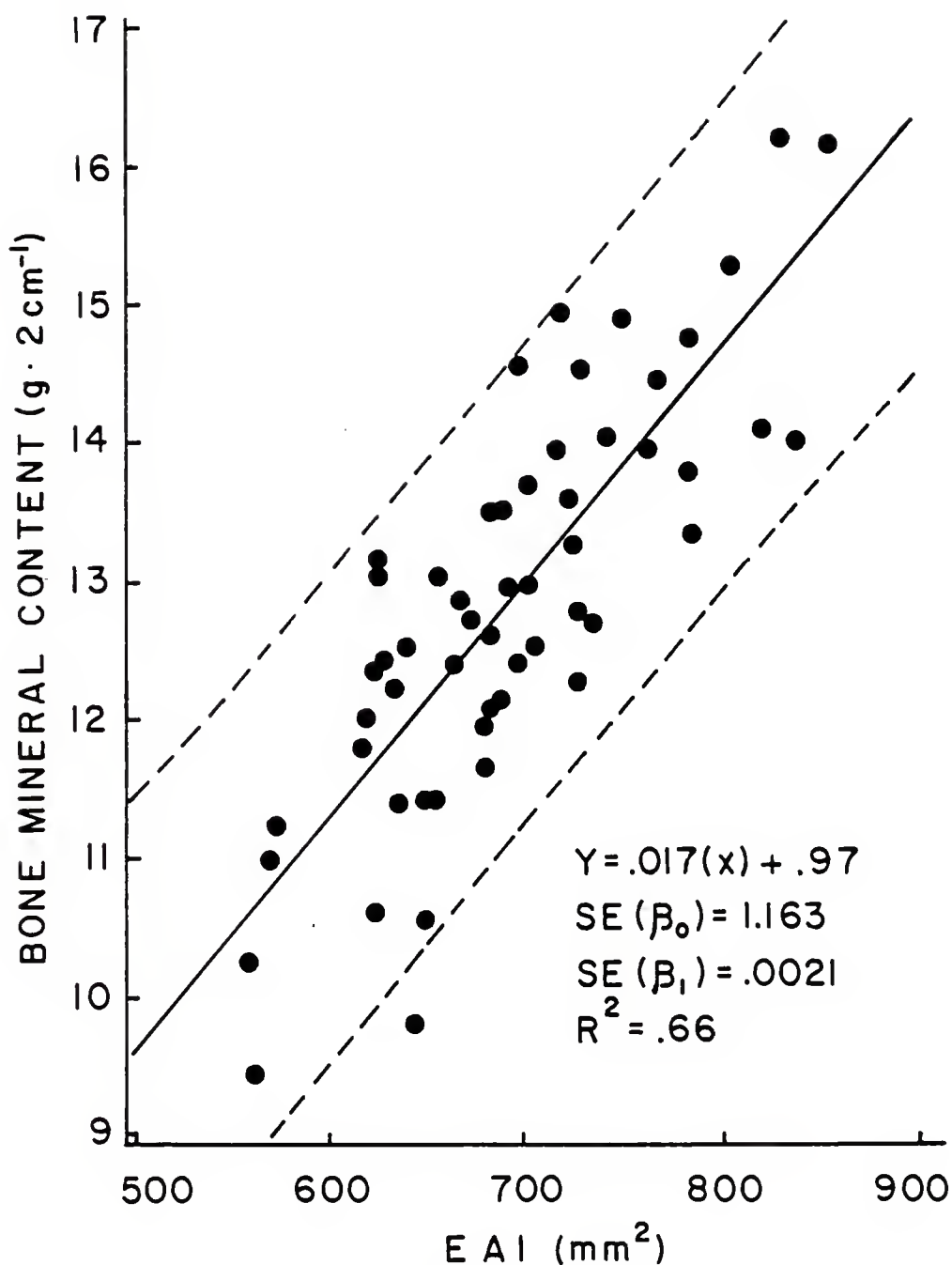


Figure 18. Relationship between bone mineral content (Y) and elliptical area index (EAI) (X) from two 2 cm third metacarpal cross-sections. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

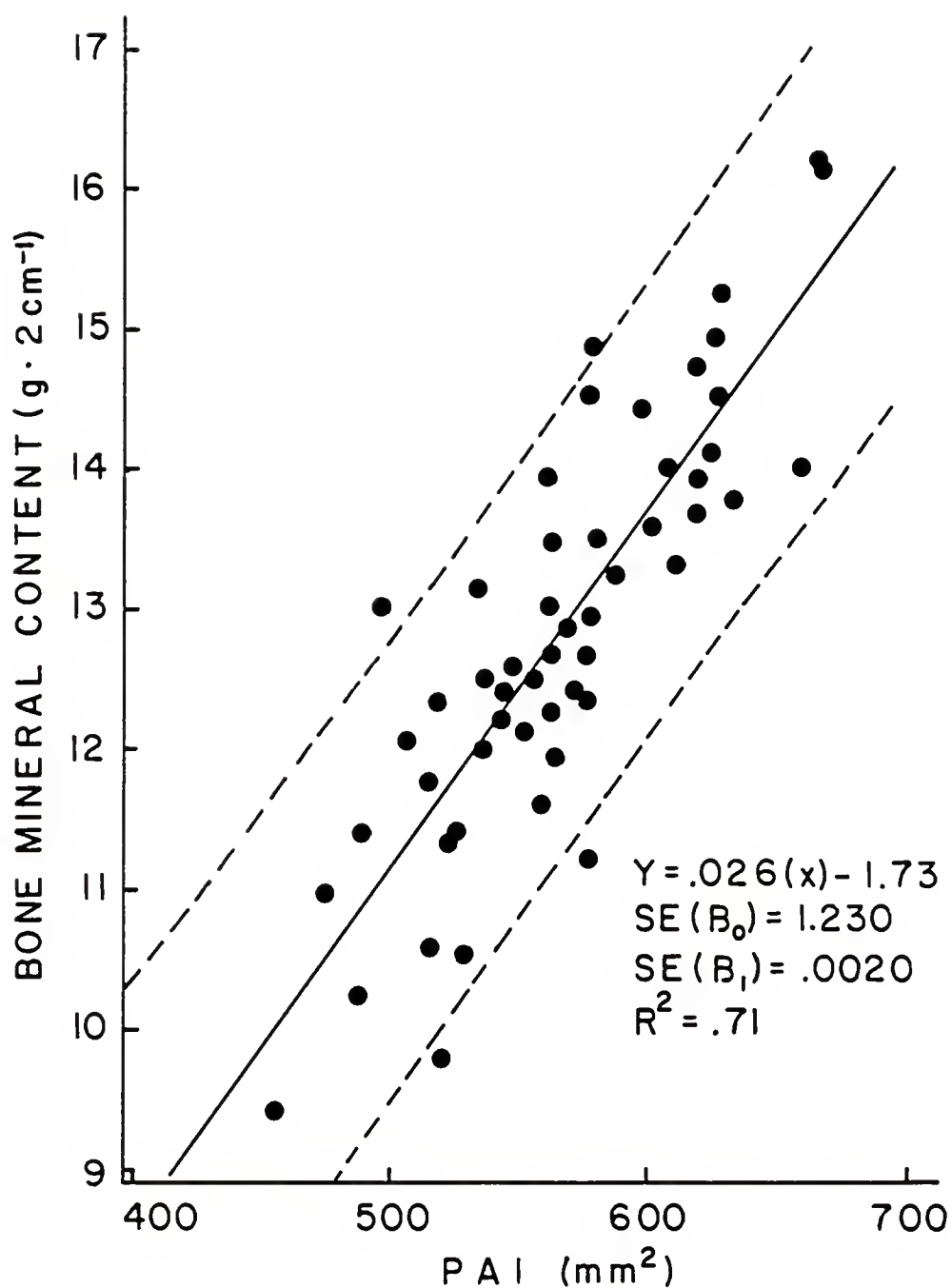


Figure 19. Relationship between bone mineral content (Y) and planimeter area index (PAI) (X) from two 2 cm third metacarpal cross-sections. Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

Table 23. Correlation coefficients between noninvasive techniques, bone mineral content (BMC), ash and various cross-sectional area indices from two 2 cm third metacarpal (McIII) cross-sections.

Item ^{a-d}	PBMC	PBMD	RBAE	U	BMC	ASH	CAI	EAI	XRI	PAI
PBMC	-									
PBMD	.947	-								
RBAE	.844	.872	-							
U	.588	.496	.559	-						
BMC	.908	.919	.967	.565	-					
ASH	.678	.712	.762	.581	.806	-				
CAI	.812	.724	.753	.644	.767	.581	-			
EAI	.860	.797	.766	.574	.812	.597	.918	-		
XRI	.770	.668	.715	.645	.715	.481	.910	.816	-	
PAI	.855	.817	.798	.476*	.842	.590	.826	.860	.751	-

^a 2 cm McIII diaphysis cross-sections removed at the midpoint and 3 cm proximal to the midpoint section.

^b Correlation coefficients based on 56 measurements.

^c PBMC = Photon absorption bone mineral content; PBMD = Photon absorption bone mineral density; RBAE = Radiographic bone aluminum equivalents; U = Ultrasound-mediolateral; CAI = Circular area index; EAI = Elliptical area index; XRI = Radiographic area index; PAI = Planimeter area index.

^d All correlations with no superscript significant ($p < .0001$).

* ($p < .001$).

can markedly improve the correlative worth of these measures.

Similar results to these have been reported elsewhere. Lawrence (1986), in a longitudinal study with horses of varying ages and breeds, reported correlations of .966 and .974 for EAI/BMC and actual cortical area/BMC, respectively. Meakim et al. (1981) have reported similar correlations of .88 for CAI and BMC and .95 for EAI and BMC.

All three noninvasive methods employed in this study were sensitive to dietary P level as differences ($p < .05$) were detected between treatment groups for all McIII locations studied (Table 22); also results obtained using DPA, RP and U followed the same pattern observed with BMC with respect to both treatment effects and McIII location differences. The results would indicate that these techniques were effective in assessing long-term bone mineral status (and thus P status) of beef cattle. Lawrence (1986), however, reported that in a short (90 d) iron supplementation trial that RP and U were not sensitive in detecting BMC changes in equine McIII that were detected by DPA and ashing.

Radiographic photometry is based on the principle that attenuation or absorption of x-rays passing through bone is proportional to the amount of light passing through a radiograph and thus related to BMC.

Peak medial, lateral and midpoint RBAE (mm, Al) determined by RP were averaged and plotted against actual BMC of 2 cm McIII diaphysis sections (Figure 20). This plot contains responses from both McIII diaphysis locations and shows little scatter of the data about the

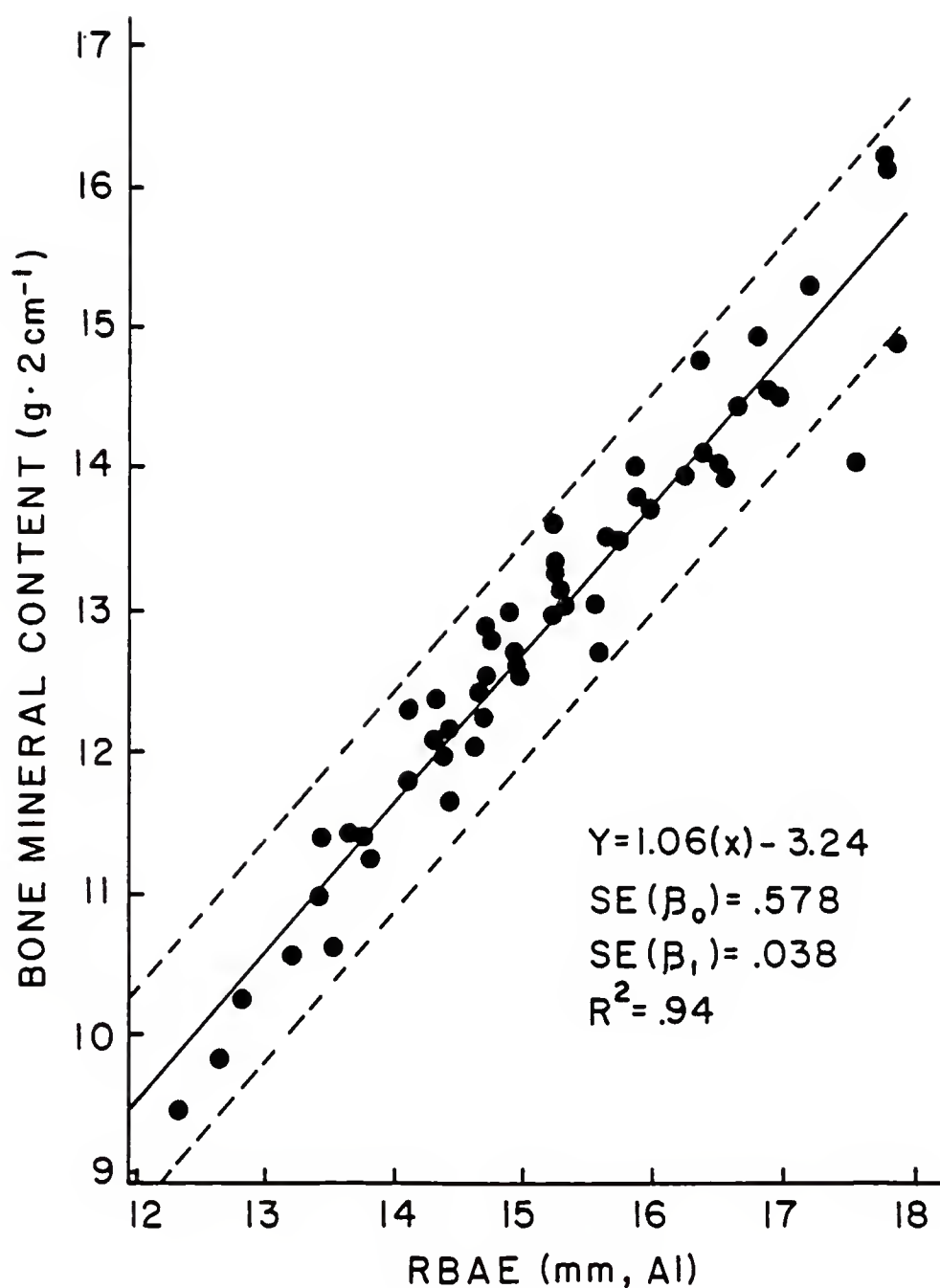


Figure 20. Relationship between bone mineral content (Y) from two 2 cm third metacarpal cross-sections and radiographic bone aluminum equivalents (RBAE) (X). Dashed lines represent 95% confidence intervals. SE (β_0) = Standard error of intercept estimate; SE (β_1) = Standard error of slope estimate.

fitted linear regression line. Omnell (1957) reported that x-rays are absorbed by Al to the same degree as bone mineral; therefore, results can be expressed in mm of Al (RBAE) or BMC. Lachman (1955) reported that with conventional radiographic techniques (i.e., without the use of a simultaneously exposed stepwedge) that a minimum Ca loss of 30% was necessary before a visual loss of BMC could be detected by radiograph. Standard errors of $\pm 6\%$ for predicting ash weight of bones using RP have been reported (Cohn, 1981).

Average RBAE was highly correlated ($r=.967$; $p<.0001$; Table 23) with BMC for all McIII locations studied. Meakim et al. (1981) reported correlations between BMC and RBAE of .94, .92 and .88 for lateral, medial and midpoint cortical aspects of equine McIII. More recently, Lawrence (1986) utilizing equine McIII reported a correlation of .956 for average RBAE and BMC, similar to that found in the present study. These correlative results between RBAE and BMC would suggest that the theoretical basis for estimating BMC via RP is sound.

The correlation between BS and average RBAE was $r=.429$ ($p<.05$; Table 24), lower than $r=.72$ reported by Lawrence (1986). As would be expected based on correlations between RBAE and BMC, a high correlation ($r=.865$; $p<.0001$; Table 24) was observed for BL and average RBAE, similar to that found for BL and actual BMC ($r=.884$; Chapter Five; Table 21).

Results of the present study would suggest that RP offers a sensitive noninvasive technique for determining BMC in bovines.

Table 24. Correlation coefficients between noninvasive techniques versus mechanical and physical properties of third metacarpals (McIII) (measured at point of failure).

Noninvasive technique ^c	McIII Property ^{a, b}								
	BL	BS	E	Strain	MIE	CAI	EAI	XRI	PAI
PBMC	.870**	.406 ⁺⁺	.198	.077	.672**	.812**	.803**	.810**	.820**
PBMD	.853**	.394	.176	.093	.654*	.771**	.748**	.758**	.807**
RBAE	.865**	.429 ⁺⁺	.286	.047	.617*	.770**	.740**	.741**	.821**
U	.588*	.499 ⁺⁺⁺	.381 ⁺⁺	-.127	.375 ⁺⁺	.723**	.635**	.678**	.579*

^a Represents break section; BL = breaking load; BS = breaking strength; E = Young's modulus; MIE = moment of inertia-ellipse; CAI = circular area index; EAI = elliptical area index; XRI = radiographic area index; PAI = planimeter area index.

^b Correlation coefficients based on 28 measurements.

^c PBMC = Photon absorption bone mineral content; PBMD = Photon absorption bone mineral density; RBAE = Radiographic bone aluminum equivalents; U = Ultrasound-mediolateral.

+ (p<.10).

++ (p<.05).

+++ (p<.01).

* (p<.001).

** (p<.0001).

Landau et al. (1985) have recently used RP to study Ca and P retention of 2-wk-old Fresian calves fed either a liquid or dry concentrate over a 39 d period. They concluded that RP was a reliable method for evaluating mineralization in bovine McIII, especially if used serially.

Photon absorption methodology initially developed by Cameron and Sorenson (1963) is based on the same principle as that of radiography, namely that the mass of bone material present is directly proportional to the amount of photon energy absorbed by bone. Smith et al. (1972) explained that the advantage of PA over radiographic techniques was the use of a monochromatic source in PA as opposed to multiple energy levels of x-rays.

Sensitivity and accuracy of the PA technique for predicting BMC have been reported in several species, including chickens (Meyer et al., 1968), dogs (Wentworth et al., 1971), rabbits (Myers et al., 1979) and horses (Jeffcott, 1985; Lawrence, 1986). Accuracy of this method for predicting BMC is reported to be within 3% (Cameron and Sorenson, 1963).

Figures 21 and 22 graphically represent the relationships between BMC and DPA estimated BMC (PBMC, $\text{g} \cdot 2.25 \text{ cm}$) and BMC and DPA bone mineral density (PBMD, $\text{g} \cdot \text{cm}^{-2}$) for all McIII sections tested, respectively. Both plots indicate a good fit of the data with little scatter around the fitted linear regression line. The correlation between BMC and PBMC was high ($r=.908$; $p<.0001$) as was the correlation between BMC and PBMD ($r=.919$; $p<.0001$) (Table 23).

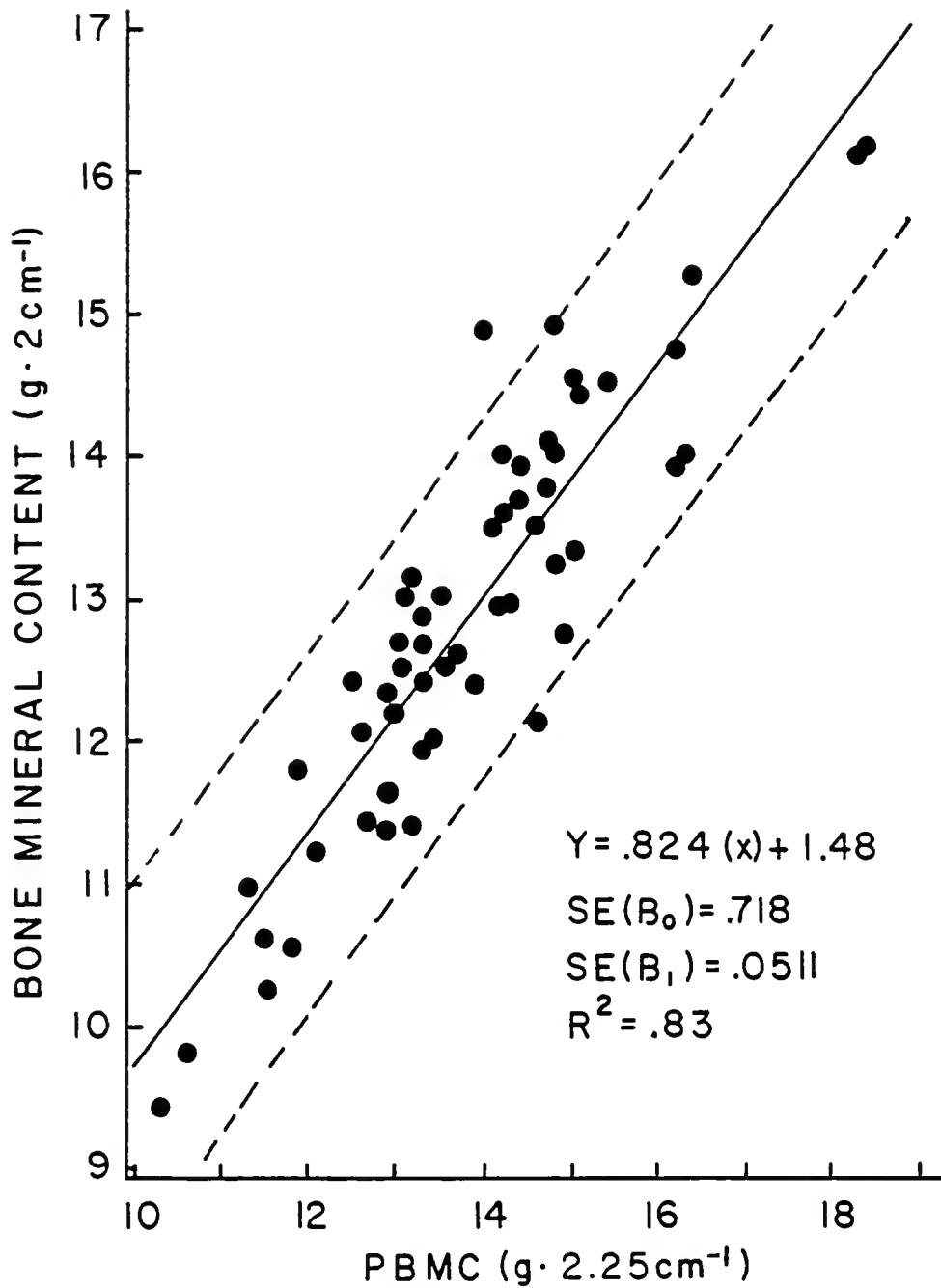


Figure 21. Relationship between bone mineral content (Y) from two 2 cm third metacarpal cross-sections and photon absorption bone mineral content (PBMC) (X). Dashed lines represent 95% confidence intervals. SE (β_0) = Standard error of intercept estimate; SE (β_1) = Standard error of slope estimate.

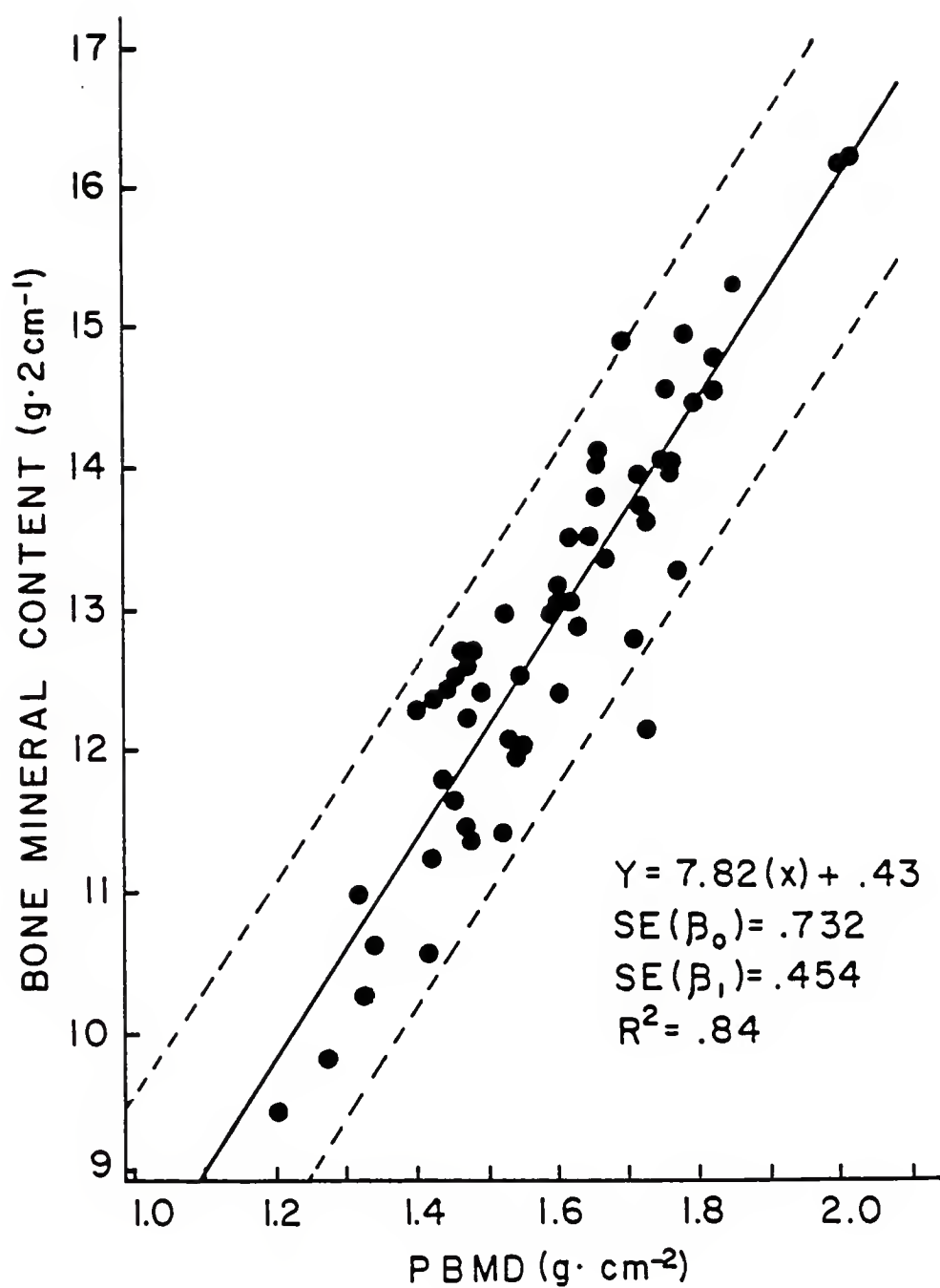


Figure 22. Relationship between bone mineral content (Y) from two 2 cm third metacarpal cross-sections and photon absorption bone mineral density (PBMD) (X). Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

Wentworth et al. (1971) and Lawrence (1986) have reported correlations over .99 between bone ash weight and photon absorption. The slightly lower correlation observed between BMC and DPA parameters in this study may be related to increased BMC (and density) in McIII sections 3 cm proximal to the McIII midpoint being out of the linear range of calibration standards used. The DPA unit used in this study (Lunar Corp., Model DP3) is calibrated to values typically seen in human bones, mainly vertebrae; however, bovine McIII are somewhat denser than human bones, possibly causing a loss of accuracy in estimating BMC. Even with this possible problem, the photon absorption unit provided a direct estimate of BMC which was very close to actual BMC (Table 22). Jeffcott (1985) has indicated that a commercially produced photon absorption unit for large animal use¹ will soon be available and may alleviate such problems.

The previous discussion may also explain the higher correlation observed between BMC and average RBAE than BMC and either DPA estimate. The Al stepwedge used as a reference standard in RP provided a range of densities within which all McIII fell. It should also be kept in mind that excised tissue free McIII were used in this study. Photon absorption use in vivo is designed to account for soft tissue effects whereas this is not true with RP, which is most effectively utilized in vivo in conjunction with bones with little soft tissue cover.

¹ Norland Digital Bone Densitometer Model 2781 Large Animal Screening System, Norland Corp., Fort Atkinson, WI.

The correlation between PBMC and BS ($r=.406$; $p<.05$; Table 24) was similar to that observed for actual BMC and BS ($r=.462$; Chapter Five; Table 21). Lawrence (1986) reported a correlation between these variables of .63 in McIII from horses of varying ages and breeds. Correlations between PBMC and BL ($r=.870$; $p<.0001$) and PBMD and BL ($r=.854$; $p<.0001$) (Table 24) were high and approached the correlation between actual BMC and BL ($r=.884$; Chapter Five; Table 21).

Photon absorptiometry has also recently been used by Holmberg et al. (1985) to monitor BMC changes in coccygeal vertebrae of dairy cattle during lactation and aid in studying parturient paresis. Although there was great individual variation among cows, BMC fluctuations during lactation were characterized in a typical pattern. They revealed that decreases in BMC as lactation proceeds are quite slow with the lowest values occurring 3 to 6 mo after parturition.

Ultrasonic techniques are based on the principle that sound waves travel through bone with a velocity that represents the material and structural properties of bone (Gerlanc et al., 1975). The relationship between BMC and ultrasound velocity is shown in Figure 23. More scatter was observed in the data with respect to ultrasounds ability to predict BMC than was observed with either DPA (Figure 21) or RP (Figure 20). The correlation between U and BMC was $r=.565$ ($p<.0001$; Table 23). This correlation is considerably lower than that of .92 recently reported by Lawrence and Ott (1985) between

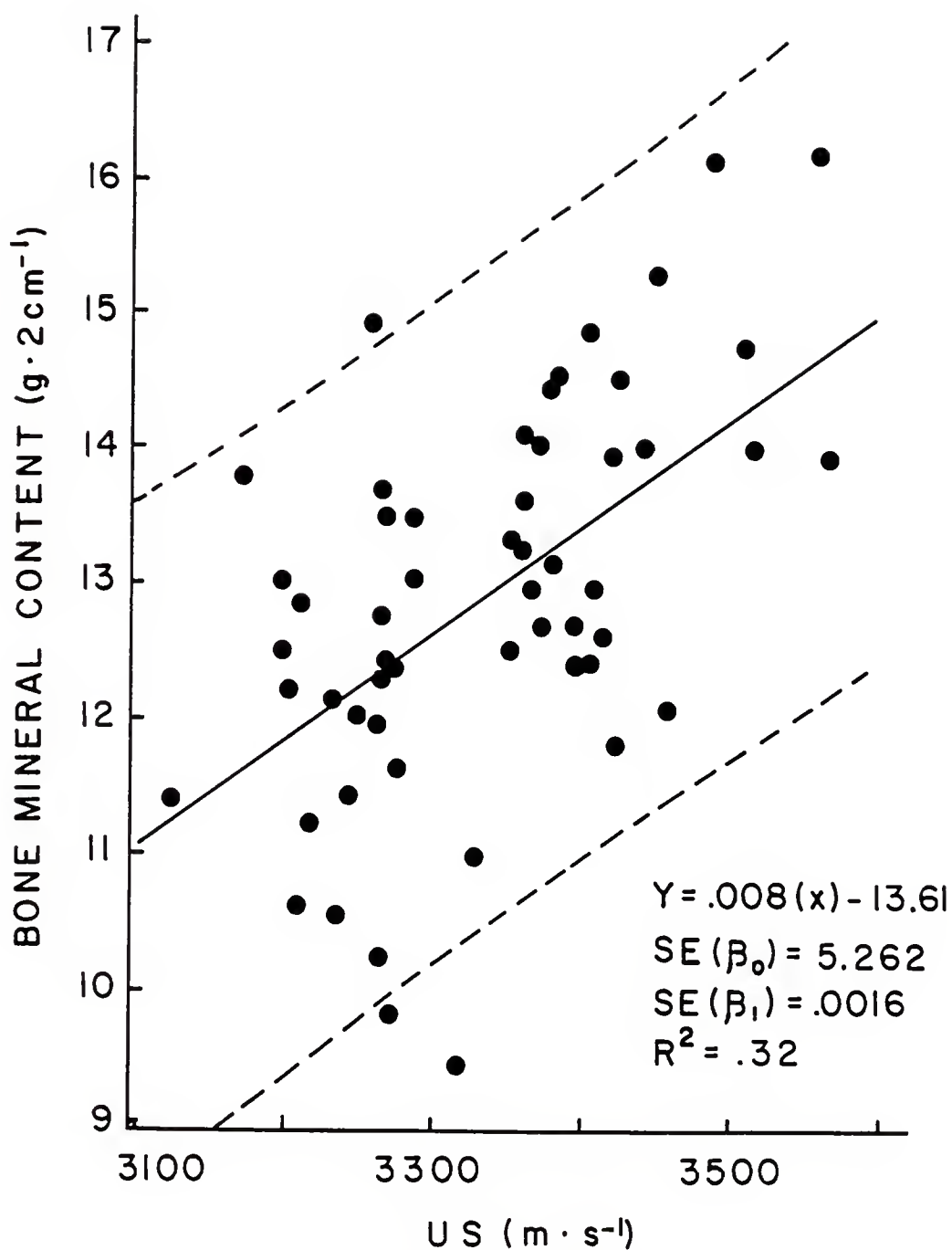


Figure 23. Relationship between bone mineral content (Y) from two 2 cm third metacarpal cross-sections and mediolateral ultrasonic velocity (US) (X). Dashed lines represent 95% confidence intervals. $SE(\beta_0)$ = Standard error of intercept estimate; $SE(\beta_1)$ = Standard error of slope estimate.

U and BMC in 25 pairs of McIII from equine cadavers of varying breeds and ages. Bokman et al. (1983) measured ultrasonic velocities at the midpoint of McIII from eight Angus cows and eight Hereford heifers and reported a correlation of .63 between bone density and U. The relatively low correlation of BMC and ultrasonic velocity in the present trial was not expected because apparent velocity of sound waves have been reported to be directly related to bone mass (Jeffcott, 1985).

None of the techniques previously discussed (i.e., photon absorption and radiographic photometry) that estimate bone mineral density consider the architectural framework in which the inorganic mineral fraction is housed. Breaking strength (and other physical properties of bone) is a function of the biphasic composite of mineral and collagen (Carter and Spengler, 1978), which are not detected by gamma-rays or x-rays, but which affect sound transmission. The relationship between U and BS is based on its association with bone density and modulus of elasticity (E) (Abendschein and Hyatt, 1972). These authors (Abendschein and Hyatt, 1970) studied the relationship between E and U in standardized specimens of human cortical bone. The E of bone has been studied extensively in its relationship to the functional capacity of bone and directly related to its BS.

Glade et al. (1986) reported BS calculated from sound velocity measurements in equine McIII were within 2% of BS measured mechanically ($r=.91$). They concluded that assessment of structural integrity, quality, change and development in long bones may be best

measured by U instead of techniques which measure mineral parameters of bone only. Lawrence and Ott (1985) reported a correlation of $r=.82$ between sound velocities through soft tissue free McIII from equine cadavers and their measured BS. Lawrence (1986) has also reported a correlation of $r=.82$ between mechanically measured E and U.

The correlation between BS and U in the present study was low ($r=.499$; $p<.01$; Table 24). Although this correlation is lower than those reported by other researchers, it was higher than those reported between BS and other noninvasive techniques studied. The correlation between E and U was low in the present study ($r=.381$; $p<.05$; Table 24) which is not consistent with the findings of Abendschein and Hyatt (1972). Breaking load and U results yielded a correlation of $r=.588$ ($p<.001$); Table 24) between these variables, which is similar to the correlation observed between BMC and U ($r=.565$; Table 23).

The actual path sound waves take through the bone has not been determined. In U data discussed above, ultrasonic velocity was calculated using the apparent (mediolateral) velocity method described by Pratt (1980). In order to better approximate the "path" sound takes as it passes through bone, velocity was calculated using the area indices (i.e., XRI, CAI, EAI and PAI). This method was first used by Lawrence et al. (1986). Correlations were higher between BMC and U as well as BL and U when cross-sectional area was used to describe the distance traveled by generated sound pulses.

The more accurate the estimate of area (PAI > EAI > CAI > XRI), the higher the correlation (Table 25). When expressing ultrasonic velocities in these terms, correlations between BMC and U (and U and BL) approached correlations found between BMC and the other noninvasive techniques evaluated. The relationships between BMC and cortical area ultrasonic velocities are shown in Figures 24, 25, 26 and 27. Compared with the relationship between BMC and U (Figure 23), there is much better fit of the data using the cortical area technique to estimate BMC. It should be noted, however, that accurate area ultrasonic velocity had lower correlations with BS than mediolateral U (Table 25). Similar results to these with respect to lower BS correlations were reported (Lawrence et al., 1986) when using accurate area ultrasound. No explanation has been or can be offered for this finding.

Conclusions

Results of this study suggest that all three noninvasive techniques used were sensitive to dietary P level in this long-term study and would aid in objective assessment of bone mineral status (and thus P status) in bovines. All three noninvasive techniques studied clearly distinguished the treatment groups as to level of dietary P they received. Also, these techniques showed similar trends with respect to actual BMC, both between treatment groups as well as between McIII locations examined. These techniques were all apparently useful in predicting BMC in McIII of heifers in this

Table 25. Correlation coefficients between ultrasound versus bone mineral content and mechanical properties of third metacarpals.

Item ^c	Ultrasound Velocity Measurement ^{a, b}				
	U	CIR-US	ELL-US	XRAY-US	PLAN-US
BMC	.565**	.810**	.835**	.775**	.854**
BL	.588*	.618*	.698**	.672**	.737**
BS	.499 ⁺⁺⁺	.329 ⁺	.225	.492 ⁺⁺⁺	.342 ⁺
E	.381 ⁺⁺	.310	.198	.414 ⁺⁺	.251
Strain	-.127	-.132	-.074	-.145	-.046

^a U = Mediolateral diameter ultrasound; CIR-US = Circular area index ultrasound; ELL-US = Elliptical area index ultrasound; XRAY-US = Radiographic area index ultrasound; PLAN-US = Planimeter area index ultrasound.

^b Correlation coefficients between BMC vs ultrasound velocity measurements and BL, BS, E and strain vs ultrasound velocity measurements based on 56, 28, 28, 28 and 28 measurements, respectively; BMC correlations coefficients are for two 2 cm section; mechanical properties correlation coefficients are only for point of failure.

^c BMC = Bone mineral content; BL = Breaking level; BS = Breaking strength; E = Young's modulus.

⁺ (p<.10).

⁺⁺ (p<.05).

⁺⁺⁺ (p<.01).

^{*} (p<.001).

^{**} (p<.0001).

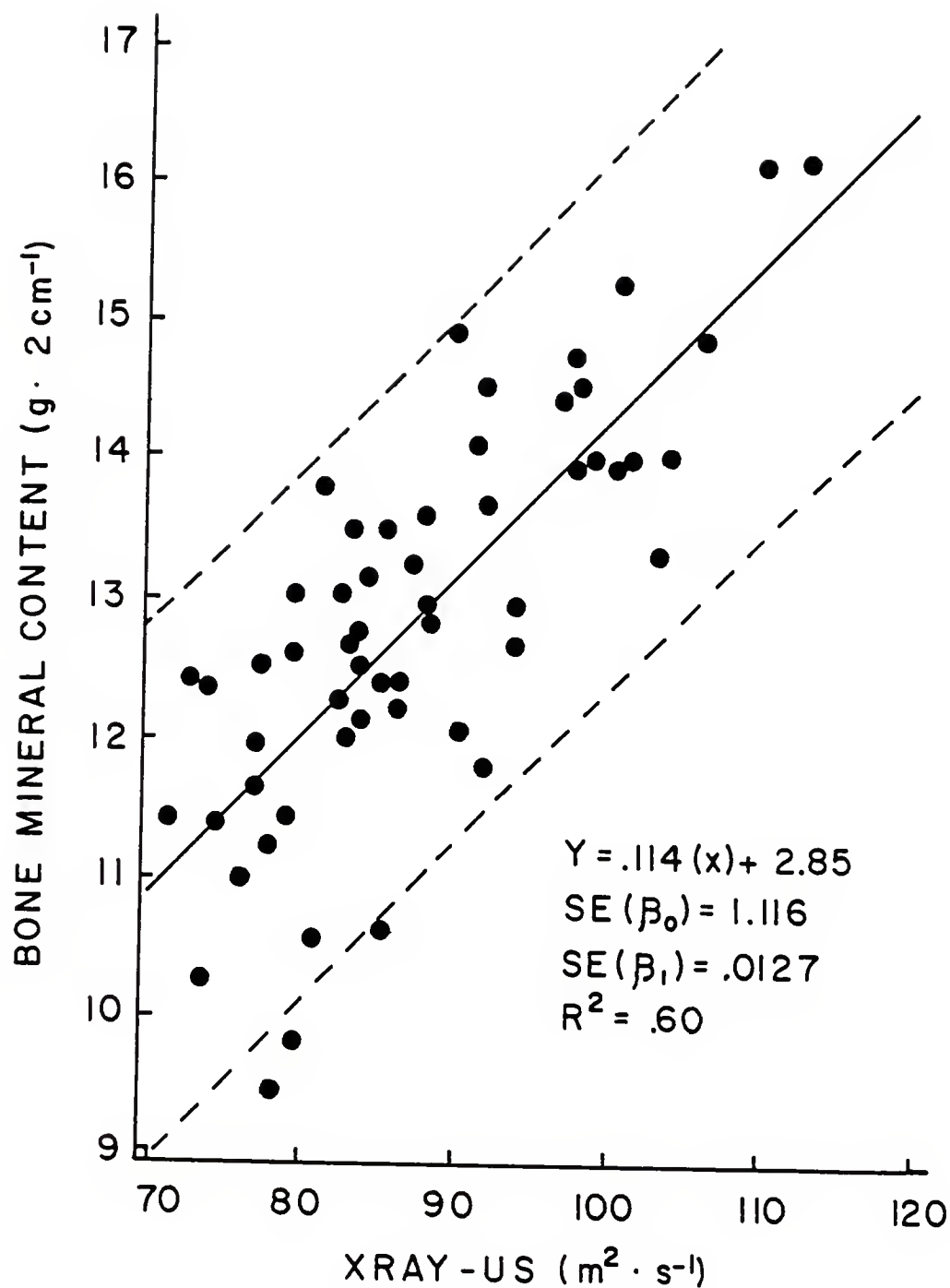


Figure 24. Relationship between bone mineral content (Y) and radiographic area index ultrasonic velocity (XRAY-US) (X). Dashed lines represent 95% confidence intervals.
 $SE(\beta_0)$ = Standard error of intercept estimate;
 $SE(\beta_1)$ = Standard error of slope estimate.

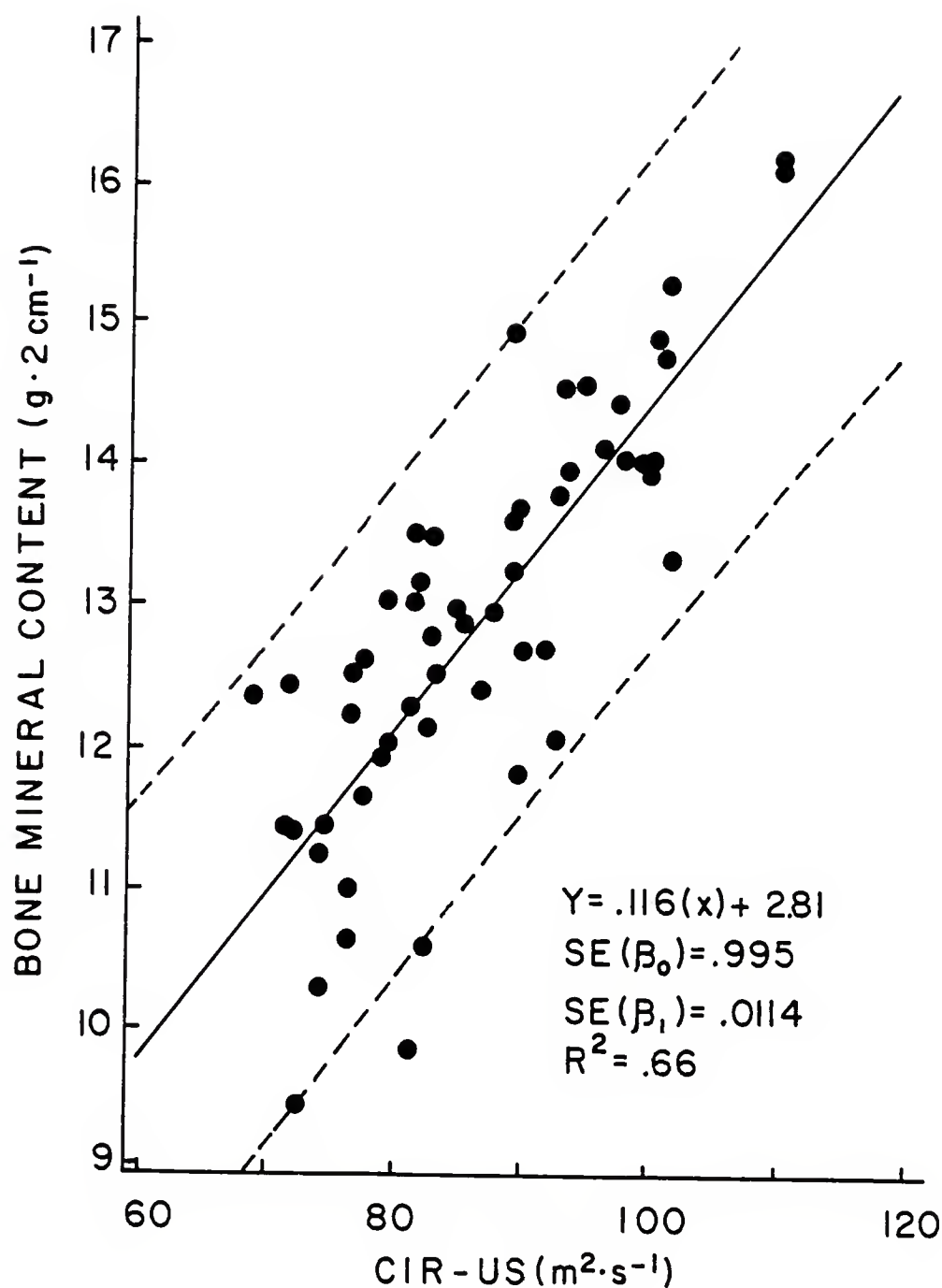


Figure 25. Relationship between bone mineral content (Y) and circular area index ultrasonic velocity (CIR-US) (X). Dashed lines represent 95% confidence intervals.
 $SE(\beta_0)$ = Standard error of intercept estimate;
 $SE(\beta_1)$ = Standard error of slope estimate.

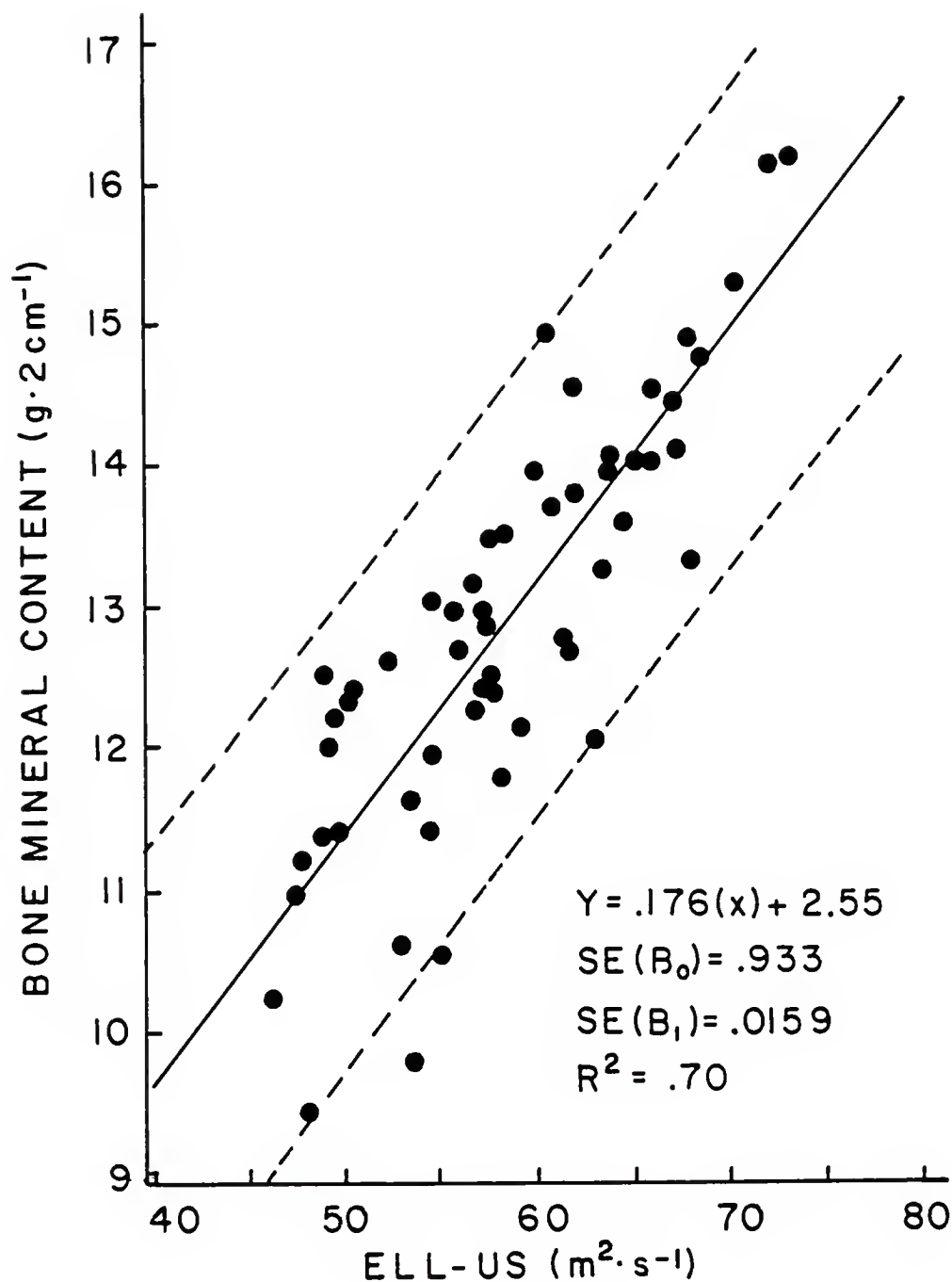


Figure 26. Relationship between bone mineral content (Y) and elliptical area index ultrasonic velocity (ELL-US) (X). Dashed lines represent 95% confidence intervals. SE (β_0) = Standard error of intercept estimate; SE (β_1) = Standard error of slope estimate.

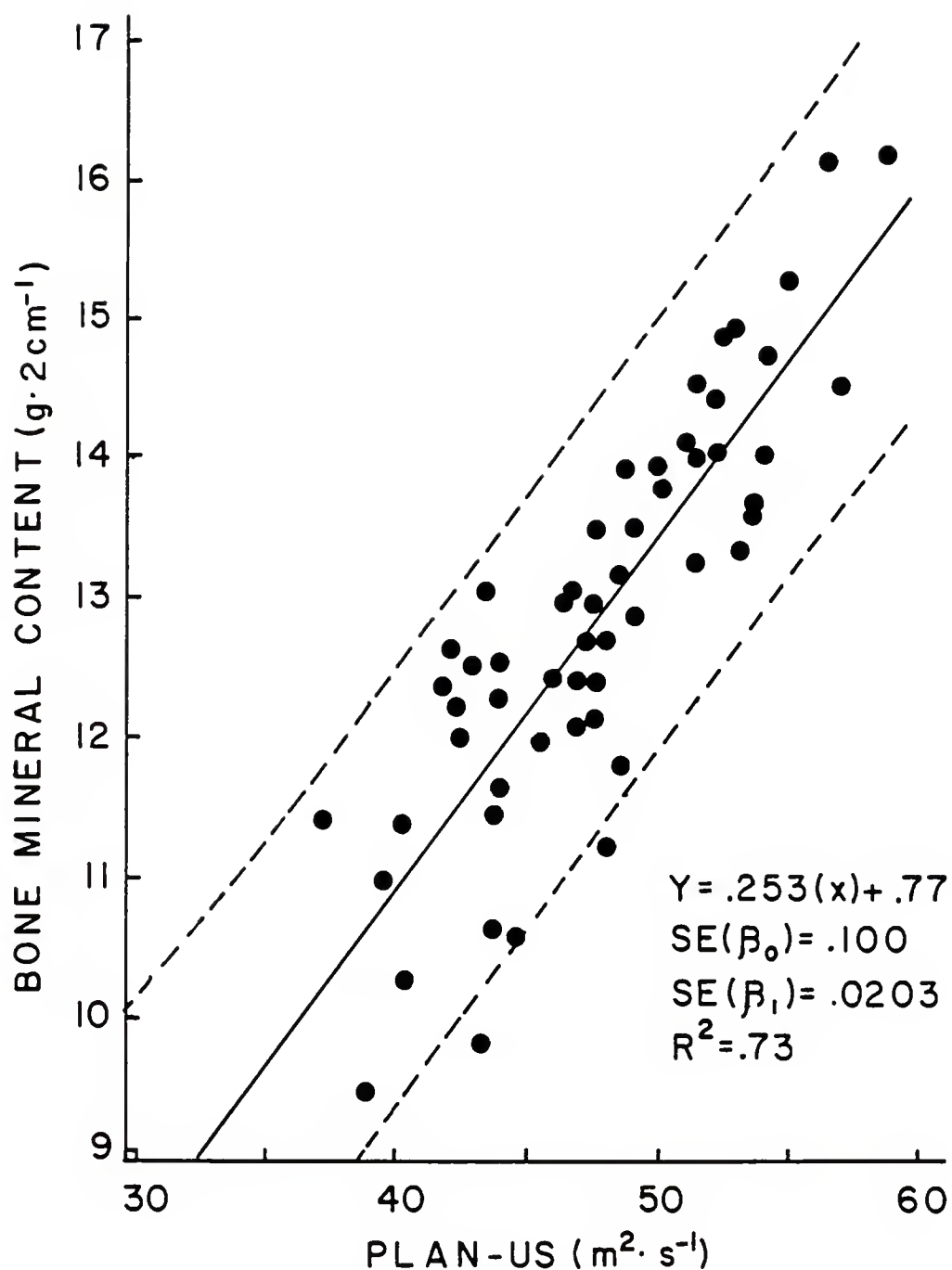


Figure 27. Relationship between bone mineral content (Y) and planimeter area index ultrasonic velocity (PLAN-US) (X). Dashed lines represent 95% confidence intervals.
 $SE(\beta_0)$ = Standard error of intercept estimate;
 $SE(\beta_1)$ = Standard error of slope estimate.

study; however, their utility in predicting bone strength properties of McIII is unclear. It is interesting that even though correlations between these techniques and bone strength properties are low compared with results of other researchers, the confidence range with which one could predict a given strength parameter using one of these techniques herein is narrower than those reported by other research groups (e.g., Lawrence and Ott, 1985).

Results of this trial would surely indicate that further investigation in studying BMC and bone strength predictive capabilities of these techniques in a longitudinal study for serially monitoring bone development in bovines is needed. Refinement of the data base would perhaps allow new insights into complex bone kinetics with respect to nutrient requirements for growth and reproduction in these animals--as well as providing an objective diagnostic tool for assessing an animal's bone mineral status at any given time nondestructively.

CHAPTER SEVEN SUMMARY AND CONCLUSIONS

An experiment was conducted to study the effect of two levels of dietary phosphorus (P) (either .12 or .20%, dry basis) on growth and reproductive performance of growing beef heifers as well as to evaluate various parameters for use as objective and reliable indicators of P status in these animals. Parameters evaluated included serum and plasma inorganic and whole blood total P concentration, various chemical and physical properties of 12th rib, coccygeal vertebrae and third metacarpals, as well as fecal, hair and salivary P concentrations. Additionally, three noninvasive techniques for assessing bone density in vivo, dual photon absorptiometry (DPA), radiographic photometry (RP) and ultrasound (U), were studied to determine their potential for assessing P status and their ability to predict both bone mineral content (BMC) and bone strength in the bovine.

The experiment ranged from 525 to 772 d in length depending on slaughter date of individual animals. The experiment consisted of a P depletion phase (270 d) followed by an adaptation period to the basal P supplementation diet (10 d) and a subsequent P supplementation phase (ranging from 245 to 492 d in length; common experimental endpoint was day 245 of P supplementation phase for nonpregnant and 3 wk postpartum for pregnant heifers).

Fifteen weaned Angus heifers (7 to 8 mo of age; 160 ± 3 kg, initially) were allowed ad libitum intake of a low P diet ($.10\%P$, dry basis) during the P depletion phase. A 10 d adaptation period to the basal P supplementation diet followed the P depletion phase. In the P supplementation phase of the experiment, heifers (averaging 210 ± 6 kg) were allotted randomly ($7 \text{ animals} \cdot \text{group}^{-1}$) to one of two dietary levels of P: 1) continuation of low P (LP) basal diet containing $.12\%P$ (dry basis), or 2) high P (HP) diet, consisting of basal diet supplemented to provide $.20\%$ total P (dry basis). Heifers were group fed ad libitum their respective treatment diets for the initial 210 d of the P supplementation phase. Feed intake was then restricted in an attempt to limit body condition in order to minimize potential calving difficulties. Heifers were bred naturally via 120 d exposure to a single bull during the P supplementation phase. Ovarian activity was monitored by rectal palpation over this period. All nonpregnant heifers were slaughtered following the final palpation (day 245 of P supplementation phase). Remaining pregnant heifers were maintained until 3 wk postpartum at which time they were slaughtered.

Samples were obtained periodically throughout the experiment; these included blood serum, blood plasma, whole blood, 12th rib bone biopsies, hair, feces and saliva. Serum, plasma and whole blood samples were also obtained from heifers and calves at parturition and 3 wk postpartum; colostrum and milk samples were also collected at these times. Samples collected at slaughter included left and right third metacarpals (McIII) and 12th ribs, coccygeal vertebrae V, VI

and VII, liver, kidney, heart and longissimus dorsi muscle. Feed samples were also collected throughout the experimental period.

Average daily gain of heifers during the P depletion phase was $.19 \text{ kg}\cdot\text{d}^{-1}$. Signs of pica, exhibited as excessive wood chewing, was observed in all heifers during the P depletion phase; however, this behavior was not exhibited in either treatment group during the P supplementation phase. During the 210 d ad libitum feeding period of the P supplementation phase total gain and ADG were greater ($p<.01$) for HP than LP heifers (257 vs 205 kg and 1.22 vs $.98 \text{ kg}\cdot\text{d}^{-1}$, respectively). These results indicate a 20% benefit to P supplemented heifers for these parameters. The HP heifers also consumed 11% more feed and were 10% more efficient in its utilization than LP heifers during this same period.

No normal estrous behavior was observed in any heifer prior to the initial palpation (day 85 of the P supplementation phase). All heifers with the exception of one from the HP group were classified as having prepubertal uteri at the initial palpation. No heifers were found to possess a corpus luteum initially (except the HP heifer with normal cycling uterine tone). By the third palpation (day 148 of the P supplementation phase) six heifers in each group were regarded as having normal uterine tone and also possessed CL. No differences ($p>.10$) were found in ovarian volumes between treatment groups. A cubic trend ($p<.01$) was observed for total ovarian volumes over the course of palpation. Five of seven heifers in the HP group were found to be pregnant and six of seven in the LP group. Birth

weight of calves were greater ($p < .10$) from HP than LP dams. Calves from HP dams had numerically greater ADG than LP calves; however, all calves from LP dams survived the 21 d postpartum period compared to three from HP dams (one was stillborn; one died unrelated to treatment effects).

Serum inorganic P and whole blood P concentration exhibited no time trend ($p > .10$) during the P depletion phase, whereas plasma inorganic P displayed a cubic trend ($p < .05$). During the P supplementation phase, a treatment*period interaction was found for both serum ($p < .05$) and plasma ($p < .10$) inorganic P. Whole blood P however showed no treatment*interaction ($p > .10$) and was not affected by dietary P level ($p > .10$). A quadratic time trend ($p < .01$) was observed for whole blood P during this phase.

Serum and plasma inorganic P concentrations were greater ($p < .01$) in HP than LP heifers during the 21 d postpartum period; whole blood P exhibited a treatment*period interaction ($p < .05$) during this period. Inorganic P concentration also increased in serum ($p < .10$) and plasma ($p < .05$) from parturition to 3 wk postpartum.

Calf serum and plasma inorganic P concentrations were not affected ($p > .10$) by dietary P level of the dam; however, both increased ($p < .01$) from day of birth to 3 wk postpartum. In contrast, calf whole blood P was affected by dietary P level of the dam ($p < .01$) and also increased ($p < .05$) from birth to 3 wk postpartum.

Dietary P level had no effect ($p > .10$) on liver, kidney, heart or muscle P concentration in samples collected at slaughter. Fecal and hair P concentrations were not affected by dietary P level ($p < .01$);

however, fecal P concentration was numerically greater in HP heifers during all sampling periods (which was not true of hair P concentrations). Saliva P levels were greater ($p < .05$) in HP than LP heifers over the periods sampled.

A dietary P level*sampling period interaction ($p < .05$) revealed that percent ash of 12th rib cortical bone was different between HP and LP heifers (61.9 vs 55.7%, respectively) on day 120 of the P supplementation phase but not on day 294 of this same period (63.8 vs 63.1%, respectively). Twelfth rib bone density was greater ($p < .05$) in HP heifers and also increased ($p < .01$) from day 120 to 294 of the P supplementation phase. Phosphorus content of 12th rib cortical bone expressed on a unit volume basis ($\text{mg}\cdot\text{cm}^{-3}$) was greater ($p < .01$) in HP heifers and increased ($p < .01$) over the two collections. Similar results were obtained for Ca and Mg when expressed on a unit volume basis; however, when expressed on an ash basis, these minerals did not behave in a like manner as would be expected based on bone calcification and resorption processes. Breaking load of 12th ribs obtained at slaughter was greater ($p < .05$) in HP heifers, indicating a greater mass of bone mineral in the ribs of these animals and agreeing with results from in vivo sampling.

Whole coccygeal vertebrae V, VI and VII obtained at slaughter indicated that HP heifers had a 23, 25 and 29%, respectively, greater ($p < .05$) amount of bone mineral mass present. Bone density was also greater ($p < .01$) in HP heifers for all vertebrae studied. Phosphorus, Ca and Mg concentrations expressed on a unit volume basis were also greater ($p < .01$) in HP than LP heifers in all vertebrae.

Whole McIII bone mineral density ($\text{g}\cdot\text{cm}^{-2}$) and bone mineral content (g) determined using DPA were greater ($p<.01$) in HP than LP heifers (1.61 vs 1.44 and 129.8 vs 114.3, respectively). It was also observed from DPA scans that LP heifers had bone loss at the midshaft of McIII, an area usually less labile, more stable, mature compact bone; whereas no such loss was observed in HP heifers.

Breaking load (BL) was greater ($p<.05$) in McIII from HP heifers. This is indicative of more bone mineral mass at the point of failure. To this point, HP heifers had greater ($p<.01$) BMC in the 2 cm of the McIII diaphysis cross-sections removed at midpoint than LP heifers (12.6 vs $11.2 \text{ g}\cdot 2 \text{ cm}^{-1}$, respectively). The correlation between BL and BMC of the 2 cm midpoint section was $r=.884$ ($p<.0001$). Planimeter, elliptical, circular and radiographic cortical area estimates of the 2 cm McIII diaphysis cross-sections removed at the midpoint of the bone all indicated that McIII cross-sections from HP heifers had greater cortical area ($p<.05$). All cross-sectional area indices were good predictors of BMC with the highest correlations observed for elliptical ($r=.812$; $p<.0001$) and planimeter ($r=.842$; $p<.0001$) cortical estimates.

Breaking strength (BS) or force per unit area of material, which takes into account both size and shape of the bone, was greater ($p<.05$) in McIII from HP heifers. This would indicate McIII bone material of HP heifers was of a higher quality than LP heifers. Percent bone ash of the 2 cm McIII diaphysis cross-section removed at the break point was greater ($p<.01$) in HP than LP heifers (68.0 vs 67.2, respectively); however, no differences ($p>.10$) in P, Ca or Mg

content expressed on an ash basis were observed between treatment groups. The correlation between BS and BMC was $r=.462$ ($p<.05$); the correlation between BS and percent ash was $r=.368$ ($p<.10$).

Young's modulus was also greater ($p<.10$) in McIII from HP than LP heifers, indicative of greater stiffness under normal stress as well as suggesting a greater ratio of bone mineral to protein in the bone matrix. Strain of McIII was not affected by dietary P ($p>.10$).

All three noninvasive techniques were sensitive to dietary P level as differences ($p<.05$) were detected between treatment groups for both McIII locations studied. The correlation between radiographic bone aluminum equivalents (RBAE, mm Al) estimated at the midpoint and 3 cm proximal to the midpoint on McIII using AP radiographs and BMC of the two 2 cm McIII diaphysis cross-sections removed from these points was $r=.967$ ($p<.0001$). Correlations between BS and BL with RBAE were $r=.429$ ($p<.05$) and $r=.865$ ($p<.0001$), respectively.

Dual photon absorption BMC (PBMC) and bone mineral density (PBMD) estimated at midpoint and 3 cm proximal to the midpoint on the McIII diaphysis were also highly correlated with BMC of the two 2 cm McIII diaphysis cross-sections removed at these points, $r=.908$ ($p<.0001$) and $r=.919$ ($p<.0001$), respectively. The correlation between PBMC and BS was $r=.406$ ($p<.05$), whereas the correlation between PBMC and BL was $r=.870$ ($p<.0001$), approaching the correlation between actual BMC and BL.

The correlation between mediolateral U measured at the midpoint and 3 cm proximal to the midpoint of McIII and BMC of the two 2 cm

McIII diaphysis cross-sections removed at these points was $r=.565$ ($p<.0001$). The correlation between BS and U was relatively low, $r=.499$ ($p<.01$); however, this correlation with BS was higher than those obtained from using either DPA or RP. The correlation between BL and U was $r=.588$ ($p<.001$).

Improvements in correlations between BMC and ultrasonic velocity as well as BL and ultrasonic velocity were obtained by using cortical area estimates (i.e., planimeter, elliptical, circular and radiographic area indices) for the distance traveled by generated sound pulses. The more accurate the estimate of area, the higher the correlation. Correlations between BMC and planimeter, elliptical, circular and radiographic ultrasound were $r=.854$, $.835$, $.810$ and $.779$ ($p<.0001$), respectively. Correlations between BL and planimeter, elliptical, circular and radiographic ultrasound were $r=.737$, $.638$, $.618$ and $.672$ ($p<.0001$), respectively.

Results of the present study would suggest that .12%P (dry basis) does not optimize growth response in young growing heifers in relation to heifers which received .20%P (dry basis). Heifers receiving the .20%P level also tended to consume more feed and be more efficient in its utilization.

Although P insufficiency has been reported to lower reproductive performance in beef cattle, in the present study .12%P was not detrimental to onset of puberty or conception rate. These results may suggest that for a limited time, .12%P is adequate to maintain reproductive performance in young heifers, relative to the .20% dietary P level.

Due to variability and factors which may influence blood P concentration, confidence in discerning P status would be difficult if blood P was used as a sole indicator of status. Rib bone biopsies provided a much clearer indication of P status in animals in this experiment than any other sample collected in vivo.

Chemical and physical properties of bovine bone were sensitive to the differing dietary P levels in this trial. All bones studied herein indicated that HP heifers deposited more total bone mineral mass than LP heifers. With respect to rib and vertebrae, the most sensitive measures of P status were bone density and P concentration expressed on a unit volume basis.

It would seem from results of the present trial that a combination of blood, bone and representative feed samples (which may be difficult to obtain in grazing ruminants) collected simultaneously under standardized conditions would provide good "working" indices of P status in ruminants at any given time, rather than one individual sample of any of these during the course of an investigation. For the most accurate assessment of P status, previous nutritional history of the animal must be considered.

Mechanical properties of bovine McIII which were sensitive to dietary P level indicated that the .12%P level utilized in this experiment was inadequate for optimum bone mineralization and strength of McIII based on lower BS values, relative to the .20%P level. The higher BL and increased BMC in McIII of HP heifers may be of particular interest in regards to animals in the breeding herd which are expected to maintain a given level of production under ever

changing field conditions for extended periods of time. Results of BS, E and strain suggest that McIII from HP heifers were more resilient. Resilience of McIII would be an important physiological trait of these bones under field conditions as the chance of lameness or fracture would be diminished in the range of normal stress. Sound locomotion is a necessary component in a long and useful productive life of a range cow. Critical consideration should, therefore, be given to the mechanical traits of bone when assessing P requirements of various classes of beef cattle.

Noninvasive techniques were all apparently useful in predicting BMC in McIII of growing heifers in this study; however, their ability in predicting bone strength properties is unclear. Further investigation of these techniques under field conditions in a longitudinal study is indicated with emphasis on monitoring bone mineral changes in mature animals. Use of a combination of these techniques would perhaps provide the ideal diagnostic tools necessary for serially monitoring various bone parameters and thus mineral status of beef cattle, all with the advantage of being nondestructive.

APPENDIX A
TABLES

Table 26. Influence of dietary phosphorus level on average gain·animal⁻¹ (kg·14d⁻¹) during the 210d ad libitum feeding period of the phosphorus supplementation phase.

Period ^a	Days of P Supplementation Phase ^b	Dietary Phosphorus Level ^c		
		LP	HP	SEM ^d
1	1-14	26.8	28.6	3.00
2	15-28	8.4 ^f	18.1 ^g	1.60
3	29-42	11.6 ^f	21.6 ^g	2.40
4	43-56	21.9	17.53	1.90
5	57-70	3.2 ^f	28.6 ^g	1.60
6	71-84	25.2	25.7	3.10
7	85-98	11.0	15.6	3.40
8	99-112	10.7 ^f	25.1 ^g	2.60
9	113-126	12.7	13.4	3.30
10	127-140	-6.1	-2.7	5.00
11	141-154	18.5	15.3	3.50
12	155-168	18.3	14.2	1.90
13	169-182	17.2	19.7	3.00
14	183-196	12.1	10.5	1.70
15	197-210	13.1	5.7	3.30
Total ^e	1-210	13.7	17.1	2.10

a 14d per period.

b Means based on 7 animals per treatment group.

c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

d Standard error of the mean.

e Total represents the overall period mean of average gain per animal.

f,g LP and HP means differ (p<.01).

Table 27. Dry matter feed intake (DMFI) during the 210d ad libitum feeding period of the phosphorus supplementation phase.

Period ^a	Days of P Supplementation Phase	Dietary Phosphorus Level ^b	
		LP	HP
----- DMFI (kg, DMB) ^c -----			
1	1-14	894.5	890.0
2	15-28	953.2	1035.0
3	29-42	923.6	1132.3
4	43-56	1085.0	1160.5
5	57-70	1063.2	1291.4
6	71-84	972.2	1237.3
7	85-98	1008.2	1264.5
8	99-112	987.3	1380.9
9	113-126	1145.5	1210.9
10	127-140	1067.7	1127.7
11	141-154	1139.1	1150.0
12	155-168	890.0	1015.9
13	169-182	961.4	1011.8
14	183-196	961.8	1006.8
15	197-210	1035.9	1057.7
Total ^d	1-210	15088.6	16972.7

^a 14d per period.

^b LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^c Dry matter intake (kg, DMB) represents the total intake of 7 animals per treatment group over a 14d period.

^d Total represents the dry matter intake for the entire 210d ad libitum feeding period during the P supplementation phase.

Table 28. Blood serum mineral concentrations--phosphorus depletion phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period					SEM ^c
	12/20/83 (66) ^b	2/16/83 (124)	4/13/83 (180)	5/25/83 (222)	7/6/83 (264)	
Mg ^d	2.42	2.18	2.31	2.09	2.51	.042
K ^d	24.1	16.3	17.2	19.8	24.4	1.05
Na ^e	567.9	313.6	313.1	368.6	374.8	12.66
Zn ^d	.084	.120	.136	.085	.078	.0058
Cu	.076	.070	.067	.075	.070	.0028

^a Least square means based on the following number of samples for the five periods chronologically: Mg, K, Zn and Cu--14, 10, 10, 4 and 14, respectively; Na--14, 10, 10, 4 and 12 respectively.

^b Day of P depletion phase.

^c Standard error of least square mean.

^d Quadratic period effect ($p < .01$).

^e Cubic period effect ($p < .01$).

Table 29. Blood plasma mineral concentrations--phosphorus depletion phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period				SEM ^c
	2/16/83 (124) ^b	4/13/83 (180)	5/25/83 (222)	7/6/83 (264)	
Mg ^e	2.09	2.12	2.20	2.36	.045
K ^d	17.2	15.7	19.4	22.9	.79
Na ^f	304.7	286.6	353.7	357.1	10.77
Zn ^e	.136	.102	.135	.127	.0114
Cu	.071	.067	.073	.066	.0038

^a Least square means based on the following number of samples for the four periods chronologically: Mg, Zn and Cu--10, 10, 4 and 14, respectively; K--10,9,4 and 11, respectively.

^b Day of P depletion phase.

^c Standard error of least square mean.

^d Quadratic period effect ($p < .01$).

^e Quadratic period effect ($p < .10$).

^f Cubic period effect ($p < .10$).

Table 30. Whole blood mineral concentrations--phosphorus depletion phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period		SEM ^c
	4/13/83 (180) ^b	5/15/83 (264)	
Mg ^d	2.11	1.79	.027
K	39.2	37.6	2.07
Na ^d	245.6	265.2	1.86
Zn	.433	.419	.0177
Cu	.074	.081	.0013

^a Least square means based on the following number of samples for Mg, K, Na, Zn and Cu for April 13, 1983 and May 25, 1983 collections--10 and 4, respectively.

^b Day of P depletion phase.

^c Standard error of least square mean.

^d Linear period effect (p<.05).

Table 31. Influence of dietary phosphorus level and sampling period on blood serum mineral concentrations--phosphorus supplementation phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period										SEM ^d
	10/8/83 (79) ^b		11/18/83 (120)		1/22/84 (186)		3/15/84 (238)		5/10/84 (294)		
	LP ^c	HP ^c	LP	HP	LP	HP	LP	HP	LP	HP	
Mg ^g	2.66	2.54	2.23	2.05	2.24	2.27	2.28	2.15	2.26	2.16	.076
K ^{e,h}	21.6	17.3	15.6	17.0	-	-	23.1	20.1	18.9	21.2	1.36
Na ^g	235.0	242.9	226.0	219.4	257.1	248.6	254.0	256.9	245.9	243.5	5.47
Zn ^g	.106	.122	.108	.094	.159	.159	.203	.201	.215	.219	.0073
Cu ^{f,g}	.075	.074	.071	.080	.090	.091	.088	.087	.108	.093	.0064

^a Least square means based on the following number of samples: 7 and 7 for the LP and HP groups, respectively, for all sampling periods except the May 10, 1984 period which was based on 5 and 5 for LP and HP groups, respectively, for Mg, Zn and Cu; K least square means based on 7 and 7, 7 and 7, 0 and 0, 1 and 2, and 4 and 4 samples for the LP and HP groups, respectively, for the five periods chronologically; Na least square means based on 7 and 7, 7 and 7, 4 and 2, and 4 and 4 samples for the LP and HP groups, respectively, for the five periods chronologically.

^b Day of P supplementation phase.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of least square mean.

^e Treatment effect ($p < .05$).

^f Linear period effect ($p < .01$).

^g Cubic period effect ($p < .01$).

^h Cubic period effect ($p < .05$).

Table 32. Influence of dietary phosphorus level and sampling period on blood plasma mineral concentrations--phosphorus supplementation phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period										SEM ^d
	10/8/83 (79) ^b		11/18/83 (120)		1/22/84 (186)		3/15/84 (238)		5/10/84 (294)		
	LP ^c	HP ^c	LP	HP	LP	HP	LP	HP	LP	HP	
Mg ^g	2.64	2.80	2.11	2.07	2.15	2.11	2.00	2.17	2.11	1.95	.059
K ^{e,h,i}	20.9	22.1	13.6	16.7	17.0	16.4	17.5	15.5	20.4	17.6	1.72
Na	235.3	237.6	228.4	241.1	233.7	249.9	227.5	233.5	245.4	251.1	10.94
Zn ^{g,i}	.120	.092	.093	.093	.166	.196	.205	.221	.224	.224	.0070
Cu ^f	.083	.076	.090	.084	.110	.102	.089	.088	.114	.098	.0071

^a Least square means based on the following number of samples: 7 and 7 for the LP and HP groups, respectively, for all sampling periods except the May 10, 1984 period which was based on 5 and 5 for LP and HP groups, respectively, for Mg, Zn and Cu; K least square means based on 7 and 7, 7 and 7, 7 and 7, 1 and 4, and 4 and 4 samples for the LP and HP groups, respectively, for the five periods chronologically; Na least square means based on 7 and 7, 7 and 7, 6 and 7, 3 and 6, and 4 and 5 samples for the LP and HP groups, respectively, for the five periods chronologically.

^b Day of P supplementation phase.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of least square mean.

^e Treatment effect (p<.05).

^f Linear period effect (p<.01).

^g Cubic period effect (p<.01).

^h Cubic period effect (p<.05).

ⁱ Treatment*period interaction (p<.10).

Table 33. Influence of dietary phosphorus level and sampling period on whole blood mineral concentrations--phosphorus supplementation phase.

Mineral ^a (mg·dl ⁻¹)	Sampling Period										SEM ^d
	10/8/83 (79) ^b		11/18/83 (120)		1/22/84 (186)		3/15/84 (238)		5/10/84 (294)		
	LP ^c	HP ^c	LP	HP	LP	HP	LP	HP	LP	HP	
Mg ^f	2.11	2.08	1.78	1.89	2.08	2.10	2.11	2.13	1.97	2.07	.044
K	45.6	41.8	50.2	45.0	46.0	42.0	49.6	42.4	51.2	45.3	2.45
Na ^e	264.3	246.8	249.0	241.4	240.9	247.4	256.4	247.6	261.8	262.0	8.37
Zn	.571	.543	.485	.560	.697	.566	.577	.589	.573	.687	.0582
Cu ^e	.080	.076	.077	.081	.073	.077	.082	.084	.087	.100	.0055

^a Least square means based on the following number of samples: 7 and 7, 6 and 7, 7 and 7, 7 and 6, and 5 and 5 for the LP and HP groups, respectively, for all minerals for the five sampling periods chronologically.

^b Day of P supplementation phase.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of least square mean.

^e Quadratic period effect ($p < .05$).

^f Cubic period effect ($p < .01$).

Table 34. Influence of dietary phosphorus level on concentration of liver, kidney, heart and muscle.

Mineral ^b	Tissue ^a											
	Liver			Kidney			Heart			Muscle		
	LP ^c	HP ^c	SEM ^d	LP	HP	SEM	LP	HP	SEM	LP	HP	SEM
Co ($\mu\text{g}\cdot\text{g}^{-1}$)	.15	.16	.020	.11	.12	.021	.05 ^e	.09 ^f	.010	.03	.11	.050
Mn ($\mu\text{g}\cdot\text{g}^{-1}$)	5.1	4.8	.29	3.5	2.9	.28	1.2	1.0	.10	.37	.36	.030
Cu ($\mu\text{g}\cdot\text{g}^{-1}$)	131.2	131.1	6.22	21.3	16.4	2.77	17.1	16.2	.60	7.8	7.7	.53
Zn ($\mu\text{g}\cdot\text{g}^{-1}$)	50.3 ^e	56.9 ^f	1.34	51.1	52.7	2.68	62.9	57.3	2.14	144.9 ^g	111.5 ^h	11.08
Fe ($\mu\text{g}\cdot\text{g}^{-1}$)	224.4	199.1	25.49	164.2	178.9	12.13	180.1	169.5	7.26	103.4	99.4	9.71
Mo ($\mu\text{g}\cdot\text{g}^{-1}$)	1.9	2.3	.19	.61	.65	.133	.15	.14	.020	.07	.07	.012

^a Tissues collected at slaughter; means based on 7 animals per treatment group.

^b Dry matter basis.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of the mean.

^{e,f} Means within a tissue row differ ($p < .05$).

^{g,h} Means within a tissue row differ ($p < .10$).

Table 35. Influence of dietary phosphorus level on ruminal and abomasal grab sample mineral concentrations.

Mineral ^b	Sample ^a					
	Rumen Contents			Abomasal Contents		
	LP ^c	HP ^c	SEM ^d	LP	HP	SEM
Ca (%)	1.32	1.80	.300	.63	1.0	.162
Mg (%)	.16	.18	.023	.06	.13	.026
K (%)	.86	.99	.061	.44	.64	.088
Zn ($\mu\text{g}\cdot\text{g}^{-1}$)	53.0	71.4	11.73	59.0	38.5	9.37
Mn ($\mu\text{g}\cdot\text{g}^{-1}$)	52.6	62.1	11.19	43.9	49.1	12.06
Cu ($\mu\text{g}\cdot\text{g}^{-1}$)	18.0	13.3	2.69	18.7	12.5	4.46
Co ($\mu\text{g}\cdot\text{g}^{-1}$)	.82	.89	.160	.72 ^e	1.05 ^f	.12
Mo ($\mu\text{g}\cdot\text{g}^{-1}$)	6.1	6.2	1.01	7.2	7.6	1.49

^a Means based on 5 animals per treatment group; rumen and abomasal contents were collected at slaughter.

^b Dry matter basis.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of the mean.

^{e,f} Means within a sample row differ ($p < .10$).

Table 36. Influence of dietary phosphorus level and sampling period on fecal mineral concentration.

Mineral ^b	Sampling Period ^a						SEM ^e
	11/18/83 (120) ^c		3/15/84 (238)		5/10/84 (294)		
	LP ^d	HP ^d	LP	HP	LP	HP	
Ca (%)	.98	1.05	.93	.98	.95	.96	.062
Mg (%) ^{g,h,l}	.57	.44	.35	.43	.31	.34	.033
K (%) ^{f,i,k}	.18	.39	.35	.36	.42	.29	.041
Zn (μg·g ⁻¹) ^j	137.3	126.3	144.4	143.1	116.1	120.9	11.54
Mn (μg·g ⁻¹) ^{g,j,l}	80.0	52.3	70.6	71.5	104.5	109.5	9.0
Cu (μg·g ⁻¹) ^h	19.8	22.1	22.8	25.5	27.8	33.2	1.74
Mo (μg·g ⁻¹) ^h	7.8	8.0	7.7	4.3	3.6	3.7	.75
Co (μg·g ⁻¹) ⁱ	.82	.76	.79	.96	1.19	.85	.080

^a Least square means based on the following number of samples: 7 and 5 for LP and HP groups, respectively, for November 18, 1983 collection; 6 and 7 for LP and HP groups, respectively, for March 15, 1984 collection; 5 and 5 for LP and HP groups, respectively, for May 10, 1984 collection.

^b Dry matter basis.

^c Day of P supplementation phase.

^d LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^e Standard error of least square means.

^f Treatment effect ($p < .01$).

^g Treatment effect ($p < .05$).

^h Linear period effect ($p < .01$).

ⁱ Linear period effect ($p < .05$).

^j Quadratic period effect ($p < .10$).

^k Treatment*period interaction ($p < .01$).

^l Treatment*period interaction ($p < .10$).

Table 37. Influence of dietary phosphorus level and sampling period on hair Zn concentration.

Mineral ^b	Sampling Period ^a						SEM ^e
	11/18/83 (79) ^c		3/15/84 (238)		5/10/84 (294)		
	LP ^d	HP ^d	LP	HP	LP	HP	
Zn (μg·g ⁻¹)	116.5	106.5	105.5	106.9	114.9	101.7	5.60

^a Least square means based on the following number of samples: 6 and 7 for LP and HP groups, respectively, for November 18, 1983; 7 and 7 for LP and HP groups, respectively, for March 15, 1984; 5 and 5 for LP and HP groups, respectively, for May 10, 1984.

^b Dry matter basis.

^c Day of P supplementation phase.

^d LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^e Standard error of least square means.

Table 38. Influence of dietary phosphorus level and time of sampling on milk mineral concentrations.

Mineral ^a	Time of Sampling				SEM ^c
	<u>Parturition (colostrum)</u>		<u>3-wk Postpartum (milk)</u>		
	LP ^b	HP ^b	LP	HP	
K (%) ^{e, f}	.34	.53	.67	.82	.075
Mg (%)	.29	.10	.12	.10	.069
Na (%) ^f	.26	.36	.40	.45	.031
Zn (μg·g ⁻¹) ^{f, g}	110.9	89.5	34.5	38.1	6.68
Cu (μg·g ⁻¹) ^d	1.3	2.2	2.2	3.4	.79

^a Dry matter basis.

^b LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB). Means based on 5 animals per treatment group (10 total).

^c Standard error of the mean.

^d Treatment effect ($p < .10$).

^e Treatment effect ($p < .05$).

^f Period effect ($p < .01$).

^g Treatment*period interaction ($p < .10$).

Table 39. Influence of dietary phosphorus level on liver biopsy mineral concentrations.

Mineral ^b	Dietary Phosphorus Level ^a		SEM ^d
	LP ^c	HP ^c	
Co ($\mu\text{g}\cdot\text{g}^{-1}$)	.46	.47	.081
Mn ($\mu\text{g}\cdot\text{g}^{-1}$)	7.3	5.9	.65
Cu ($\mu\text{g}\cdot\text{g}^{-1}$)	198.3	202.9	20.92
Fe ($\mu\text{g}\cdot\text{g}^{-1}$)	237.3	203.2	21.65
Zn ($\mu\text{g}\cdot\text{g}^{-1}$)	79.4	74.5	7.54
Mo ($\mu\text{g}\cdot\text{g}^{-1}$)	2.8	3.0	.27

^a Least square means based on the following number of samples: 7 and 6 for LP and HP groups, respectively. Samples collected November 18, 1983.

^b Dry matter basis.

^c LP (low phosphorus; .12%P, DMB); HP (high phosphorus; .20%P, DMB).

^d Standard error of least square mean.

APPENDIX B
RAW EXPERIMENTAL DATA

Cow Weight Data Codes

Animal = Cow number

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

W1-W28 = Cow weights (lbs) at 14d intervals (i.e., twenty-eight 14d periods)

COW WEIGHT DATA

OBS	ANIMAL	TRT	W1	W2	W3	W4	W5	W6	W7
1	168	1	384	456	484	526	570	588	656
2	169	1	464	544	548	566	602	604	652
3	170	1	546	596	610	644	682	682	726
4	171	1	481	548	566	568	622	624	664
5	172	1	424	462	494	514	570	586	644
6	173	1	450	508	524	536	594	588	646
7	174	1	494	542	562	612	664	682	754
8	276	2	396	454	482	538	556	624	668
9	277	2	434	494	526	578	630	696	720
10	278	2	444	478	532	584	636	686	748
11	279	2	456	546	580	610	656	724	778
12	280	2	466	552	594	650	680	754	800
13	281	2	562	626	594	708	736	792	886
14	282	2	462	510	558	602	646	704	776

OBS	ANIMAL	TRT	W8	W9	W10	W11	W12	W13	W14
1	168	1	686	724	750	722	754	810	832
2	169	1	666	698	736	720	766	796	856
3	170	1	746	738	780	748	778	818	846
4	171	1	698	740	726	724	772	800	854
5	172	1	674	698	752	732	782	828	866
6	173	1	662	674	714	710	762	796	848
7	174	1	780	804	814	822	850	898	910
8	276	2	708	760	784	784	840	868	904
9	277	2	802	878	908	880	918	952	1026
10	278	2	768	804	830	810	868	896	918
11	279	2	830	880	912	898	924	932	984
12	280	2	812	880	886	866	908	946	998
13	281	2	888	946	978	940	978	1014	1048
14	282	2	808	854	910	989	966	1012	1046

COW WEIGHT DATA

OBS	ANIMAL	TRT	W15	W16	W17	W18	W19	W20	W21
1	168	1	872	890	932	976	982	1028	1056
2	169	1	878	914	948	988	1002	1022	1064
3	170	1	868	896	890	928	918	948	946
4	171	1	876	892	926	954	966	994	1032
5	172	1	886	926	968	986	.	.	.
6	173	1	888	912	944	954	.	.	.
7	174	1	930	970	984	1024	1024	1042	1058
8	276	2	928	946	978	1002	998	1024	1036
9	277	2	1032	1084	1076	1074	1138	1184	1192
10	278	2	946	946	984	996	1098	1126	1146
11	279	2	1024	1034	1056	1084	.	.	.
12	280	2	1018	1024	1074	1092	1112	1114	1144
13	281	2	1066	1038	1096	1112	1206	1220	1248
14	282	2	1072	1102	1138	1152	.	.	.
OBS	ANIMAL	TRT	W22	W23	W24	W25	W26	W27	W28
1	168	1	1024	1046	1060	1078	1080	1060	1048
2	169	1	1038	1054	1074	1084	1050	1060	1028
3	170	1	934	938	950	958	960	982	946
4	171	1	1020	1028	1020	1028	1016	1028	1002
5	172	1	.	.	.	.	.	.	.
6	173	1	.	.	.	.	.	.	.
7	174	1	1048	1066	1068	1076	1058	1052	1028
8	276	2	1020	1040	1036	1050	1056	1062	1052
9	277	2	1170	1174	1196	1188	1182	1172	1142
10	278	2	1130	1152	1135	1148	1128	1138	1128
11	279	2	1118	1116	1106	1114	1108	1116	1102
12	280	2	1244	1236	1240	1252	1248	1248	1130
13	281	2							
14	282	2							

Daily Group Intake Data Codes

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

Period 1 = day 1 to 14
2 = day 15 to 28
3 = day 29 to 42
4 = day 43 to 56
5 = day 57 to 70
6 = day 71 to 84
7 = day 85 to 98
8 = day 97 to 112
9 = day 113 to 126
10 = day 127 to 140
11 = day 141 to 154
12 = day 155 to 168
13 = day 169 to 182
14 = day 183 to 196
15 = day 197 to 210

Day 1 = day 1 of period
2 = day 2 of period
3 = day 3 of period
4 = day 4 of period
5 = day 5 of period
6 = day 6 of period
7 = day 7 of period
8 = day 8 of period
9 = day 9 of period
10 = day 10 of period
11 = day 11 of period
12 = day 12 of period
13 = day 13 of period
14 = day 14 of period

Intake = daily group feed intake (lbs, DMB)

DAILY GROUP INTAKE DATA

OBS	TRT	PERIOD	DAY	INTAKE	OBS	TRT	PERIOD	DAY	INTAKE
1	1	1	1	133	76	1	6	6	167
2	1	1	1	130	77	1	6	6	143
3	1	1	1	107	78	1	6	6	147
4	1	1	1	114	79	1	6	6	164
5	1	1	1	133	80	1	6	6	143
6	1	1	1	138	81	1	6	6	171
7	1	1	1	141	82	1	6	6	137
8	1	1	1	143	83	1	6	6	132
9	1	1	1	132	84	1	6	6	132
10	1	1	1	146	85	1	7	7	173
11	1	1	1	141	86	1	7	7	200
12	1	1	1	138	87	1	7	7	137
13	1	1	1	134	88	1	7	7	131
14	1	1	1	139	89	1	7	7	132
15	1	1	1	148	90	1	7	7	132
16	1	1	1	160	91	1	7	7	133
17	1	1	1	141	92	1	7	7	133
18	1	1	1	133	93	1	7	7	133
19	1	1	1	133	94	1	7	7	133
20	1	1	1	149	95	1	7	7	140
21	1	1	1	137	96	1	7	7	147
22	1	1	1	130	97	1	7	7	138
23	1	1	1	133	98	1	7	7	133
24	1	1	1	133	99	1	7	7	132
25	1	1	1	133	100	1	7	7	132
26	1	1	1	147	101	1	7	7	132
27	1	1	1	130	102	1	7	7	133
28	1	1	1	133	103	1	7	7	133
29	1	1	1	144	104	1	7	7	133
30	1	1	1	133	105	1	7	7	133
31	1	1	1	133	106	1	7	7	133
32	1	1	1	133	107	1	7	7	133
33	1	1	1	133	108	1	7	7	133
34	1	1	1	133	109	1	7	7	133
35	1	1	1	133	110	1	7	7	133
36	1	1	1	133	111	1	7	7	133
37	1	1	1	133	112	1	7	7	133
38	1	1	1	133	113	1	7	7	133
39	1	1	1	140	114	1	7	7	133
40	1	1	1	133	115	1	7	7	133
41	1	1	1	133	116	1	7	7	133
42	1	1	1	133	117	1	7	7	133
43	1	1	1	133	118	1	7	7	133
44	1	1	1	133	119	1	7	7	133
45	1	1	1	133	120	1	7	7	133
46	1	1	1	133	121	1	7	7	133
47	1	1	1	133	122	1	7	7	133
48	1	1	1	133	123	1	7	7	133
49	1	1	1	133	124	1	7	7	133
50	1	1	1	133	125	1	7	7	133
51	1	1	1	133	126	1	7	7	133
52	1	1	1	133	127	1	7	7	133
53	1	1	1	133	128	1	7	7	133
54	1	1	1	133	129	1	7	7	133
55	1	1	1	133	130	1	7	7	133
56	1	1	1	133	131	1	7	7	133
57	1	1	1	133	132	1	7	7	133
58	1	1	1	133	133	1	7	7	133
59	1	1	1	133	134	1	7	7	133
60	1	1	1	133	135	1	7	7	133
61	1	1	1	133	136	1	7	7	133
62	1	1	1	133	137	1	7	7	133
63	1	1	1	133	138	1	7	7	133
64	1	1	1	133	139	1	7	7	133
65	1	1	1	133	140	1	7	7	133
66	1	1	1	133	141	1	7	7	133
67	1	1	1	133	142	1	7	7	133
68	1	1	1	133	143	1	7	7	133
69	1	1	1	133	144	1	7	7	133
70	1	1	1	133	145	1	7	7	133
71	1	1	1	133	146	1	7	7	133
72	1	1	1	133	147	1	7	7	133
73	1	1	1	133	148	1	7	7	133
74	1	1	1	133	149	1	7	7	133
75	1	1	1	133	150	1	7	7	133

DAILY GROUP INTAKE DATA

OBS	TRT	PERIOD	DAY	INTAKE	OBS	TRT	PERIOD	DAY	INTAKE
11	1	1	1	161	11	1	1	16	
11	1	1	1	176	11	1	1	16	
11	1	1	1	186	11	1	1	16	
11	1	1	1	196	11	1	1	16	
11	1	1	1	206	11	1	1	16	
11	1	1	1	216	11	1	1	16	
11	1	1	1	226	11	1	1	16	
11	1	1	1	236	11	1	1	16	
11	1	1	1	246	11	1	1	16	
11	1	1	1	256	11	1	1	16	
11	1	1	1	266	11	1	1	16	
11	1	1	1	276	11	1	1	16	
11	1	1	1	286	11	1	1	16	
11	1	1	1	296	11	1	1	16	
11	1	1	1	306	11	1	1	16	
11	1	1	1	316	11	1	1	16	
11	1	1	1	326	11	1	1	16	
11	1	1	1	336	11	1	1	16	
11	1	1	1	346	11	1	1	16	
11	1	1	1	356	11	1	1	16	
11	1	1	1	366	11	1	1	16	
11	1	1	1	376	11	1	1	16	
11	1	1	1	386	11	1	1	16	
11	1	1	1	396	11	1	1	16	
11	1	1	1	406	11	1	1	16	
11	1	1	1	416	11	1	1	16	
11	1	1	1	426	11	1	1	16	
11	1	1	1	436	11	1	1	16	
11	1	1	1	446	11	1	1	16	
11	1	1	1	456	11	1	1	16	
11	1	1	1	466	11	1	1	16	
11	1	1	1	476	11	1	1	16	
11	1	1	1	486	11	1	1	16	
11	1	1	1	496	11	1	1	16	
11	1	1	1	506	11	1	1	16	
11	1	1	1	516	11	1	1	16	
11	1	1	1	526	11	1	1	16	
11	1	1	1	536	11	1	1	16	
11	1	1	1	546	11	1	1	16	
11	1	1	1	556	11	1	1	16	
11	1	1	1	566	11	1	1	16	
11	1	1	1	576	11	1	1	16	
11	1	1	1	586	11	1	1	16	
11	1	1	1	596	11	1	1	16	
11	1	1	1	606	11	1	1	16	
11	1	1	1	616	11	1	1	16	
11	1	1	1	626	11	1	1	16	
11	1	1	1	636	11	1	1	16	
11	1	1	1	646	11	1	1	16	
11	1	1	1	656	11	1	1	16	
11	1	1	1	666	11	1	1	16	
11	1	1	1	676	11	1	1	16	
11	1	1	1	686	11	1	1	16	
11	1	1	1	696	11	1	1	16	
11	1	1	1	706	11	1	1	16	
11	1	1	1	716	11	1	1	16	
11	1	1	1	726	11	1	1	16	
11	1	1	1	736	11	1	1	16	
11	1	1	1	746	11	1	1	16	
11	1	1	1	756	11	1	1	16	
11	1	1	1	766	11	1	1	16	
11	1	1	1	776	11	1	1	16	
11	1	1	1	786	11	1	1	16	
11	1	1	1	796	11	1	1	16	
11	1	1	1	806	11	1	1	16	
11	1	1	1	816	11	1	1	16	
11	1	1	1	826	11	1	1	16	
11	1	1	1	836	11	1	1	16	
11	1	1	1	846	11	1	1	16	
11	1	1	1	856	11	1	1	16	
11	1	1	1	866	11	1	1	16	
11	1	1	1	876	11	1	1	16	
11	1	1	1	886	11	1	1	16	
11	1	1	1	896	11	1	1	16	
11	1	1	1	906	11	1	1	16	
11	1	1	1	916	11	1	1	16	
11	1	1	1	926	11	1	1	16	
11	1	1	1	936	11	1	1	16	
11	1	1	1	946	11	1	1	16	
11	1	1	1	956	11	1	1	16	
11	1	1	1	966	11	1	1	16	
11	1	1	1	976	11	1	1	16	
11	1	1	1	986	11	1	1	16	
11	1	1	1	996	11	1	1	16	
11	1	1	1	1006	11	1	1	16	

DAILY GROUP INTAKE DATA

OBS	TRT	PERIOD	DAY	INTAKE	OBS	TRT	PERIOD	DAY	INTAKE
1	N	7	7	210	376	N	13	12	148
2	N	7	8	202	377	N	13	13	147
3	N	7	9	14	378	N	13	14	134
4	N	7	10	74	379	N	13	15	161
5	N	7	11	97	380	N	13	16	139
6	N	7	12	86	381	N	13	17	130
7	N	7	13	133	382	N	13	18	138
8	N	7	14	167	383	N	13	19	130
9	N	7	15	14	384	N	13	20	141
10	N	7	16	82	385	N	13	21	141
11	N	7	17	16	386	N	13	22	163
12	N	7	18	11	387	N	13	23	171
13	N	7	19	220	388	N	13	24	160
14	N	7	20	134	389	N	13	25	188
15	N	7	21	155	390	N	13	26	144
16	N	7	22	177	391	N	13	27	163
17	N	7	23	164	392	N	13	28	163
18	N	7	24	139	393	N	13	29	144
19	N	7	25	103	394	N	13	30	144
20	N	7	26	98	395	N	13	31	144
21	N	7	27	201	396	N	13	32	144
22	N	7	28	173	397	N	13	33	144
23	N	7	29	184	398	N	13	34	144
24	N	7	30	206	399	N	13	35	144
25	N	7	31	161	400	N	13	36	144
26	N	7	32	212	401	N	13	37	144
27	N	7	33	185	402	N	13	38	144
28	N	7	34	140	403	N	13	39	144
29	N	7	35	195	404	N	13	40	144
30	N	7	36	170	405	N	13	41	144
31	N	7	37	145	406	N	13	42	144
32	N	7	38	174	407	N	13	43	144
33	N	7	39	220	408	N	13	44	144
34	N	7	40	199	409	N	13	45	144
35	N	7	41	188	410	N	13	46	144
36	N	7	42	162	411	N	13	47	144
37	N	7	43	173	412	N	13	48	144
38	N	7	44	182	413	N	13	49	144
39	N	7	45	190	414	N	13	50	144
40	N	7	46	163	415	N	13	51	144
41	N	7	47	171	416	N	13	52	144
42	N	7	48	174	417	N	13	53	144
43	N	7	49	197	418	N	13	54	144
44	N	7	50	193	419	N	13	55	144
45	N	7	51	175	420	N	13	56	144
46	N	7	52	180	421	N	13	57	144
47	N	7	53	164	422	N	13	58	144
48	N	7	54	186	423	N	13	59	144
49	N	7	55	168	424	N	13	60	144
50	N	7	56	191	425	N	13	61	144
51	N	7	57	197	426	N	13	62	144
52	N	7	58	210	427	N	13	63	144
53	N	7	59	222	428	N	13	64	144
54	N	7	60	200	429	N	13	65	144
55	N	7	61	149	430	N	13	66	144
56	N	7	62	190	431	N	13	67	144
57	N	7	63	175	432	N	13	68	144
58	N	7	64	155	433	N	13	69	144
59	N	7	65	110	434	N	13	70	144
60	N	7	66	201	435	N	13	71	144
61	N	7	67	188	436	N	13	72	144
62	N	7	68	237	437	N	13	73	144
63	N	7	69	151	438	N	13	74	144
64	N	7	70	141	439	N	13	75	144
65	N	7	71	162	440	N	13	76	144
66	N	7	72	149	441	N	13	77	144
67	N	7	73	149	442	N	13	78	144
68	N	7	74	150	443	N	13	79	144
69	N	7	75	148	444	N	13	80	144

Blood Serum, Blood Plasma, Whole Blood, Hemoglobin
and Hematocrit Data Codes

Animal = Cow number

Period 1 = December 20, 1982

2 = February 16, 1983

3 = April 13, 1983

4 = May 25, 1983

5 = July 6, 1983

6 = October 8, 1983

7 = November 18, 1983

8 = January 22, 1984

9 = March 15, 1984

10 = May 10, 1984

Trt 1 = Low phosphorus (.12% P, DMB)

2 = High phosphorus (.20% P, DMB)

S = Serum ($\text{mg} \cdot \text{dl}^{-1}$)

P = Plasma ($\text{mg} \cdot \text{dl}^{-1}$)

WB = Whole blood ($\text{mg} \cdot \text{dl}^{-1}$)

P = Phosphorus

Ca = Calcium

Mg = Magnesium

K = Potassium

Na = Sodium

Zn = Zinc

Cu = Copper

HB = Hemoglobin ($\text{g} \cdot \text{dl}^{-1}$)

PCV = Hematocrit (%)

BLOOD SERUM DATA										
OBS	ANIMAL	PERIOD	TRT	SP	SCA	SMG	SK	SNA	SZN	SCU
76	76	6	77	4.97	9.69	2.10	16	2.48	0.090	0.074
77	76	7	77	4.94	9.41	2.11	17	2.48	0.083	0.081
78	76	8	77	4.94	9.58	2.11	17	2.48	0.157	0.107
79	76	9	77	4.94	7.48	2.11	17	2.48	0.186	0.086
80	76	10	77	4.94	7.33	2.11	17	2.47	0.232	0.087
81	76	11	77	4.94	7.33	2.11	17	2.47	0.081	0.060
82	76	12	77	4.94	7.33	2.11	17	2.40	0.109	0.064
83	76	13	77	4.94	7.33	2.11	17	2.40	0.165	0.070
84	76	14	77	4.94	7.33	2.11	17	2.40	0.165	0.070
85	76	15	77	4.94	7.33	2.11	17	2.40	0.165	0.070
86	76	16	77	4.94	7.33	2.11	17	2.40	0.165	0.070
87	76	17	77	4.94	7.33	2.11	17	2.40	0.165	0.070
88	76	18	77	4.94	7.33	2.11	17	2.40	0.165	0.070
89	76	19	77	4.94	7.33	2.11	17	2.40	0.165	0.070
90	76	20	77	4.94	7.33	2.11	17	2.40	0.165	0.070
91	76	21	77	4.94	7.33	2.11	17	2.40	0.165	0.070
92	76	22	77	4.94	7.33	2.11	17	2.40	0.165	0.070
93	76	23	77	4.94	7.33	2.11	17	2.40	0.165	0.070
94	76	24	77	4.94	7.33	2.11	17	2.40	0.165	0.070
95	76	25	77	4.94	7.33	2.11	17	2.40	0.165	0.070
96	76	26	77	4.94	7.33	2.11	17	2.40	0.165	0.070
97	76	27	77	4.94	7.33	2.11	17	2.40	0.165	0.070
98	76	28	77	4.94	7.33	2.11	17	2.40	0.165	0.070
99	76	29	77	4.94	7.33	2.11	17	2.40	0.165	0.070
100	76	30	77	4.94	7.33	2.11	17	2.40	0.165	0.070
101	76	31	77	4.94	7.33	2.11	17	2.40	0.165	0.070
102	76	32	77	4.94	7.33	2.11	17	2.40	0.165	0.070
103	76	33	77	4.94	7.33	2.11	17	2.40	0.165	0.070
104	76	34	77	4.94	7.33	2.11	17	2.40	0.165	0.070
105	76	35	77	4.94	7.33	2.11	17	2.40	0.165	0.070
106	76	36	77	4.94	7.33	2.11	17	2.40	0.165	0.070
107	76	37	77	4.94	7.33	2.11	17	2.40	0.165	0.070
108	76	38	77	4.94	7.33	2.11	17	2.40	0.165	0.070
109	76	39	77	4.94	7.33	2.11	17	2.40	0.165	0.070
110	76	40	77	4.94	7.33	2.11	17	2.40	0.165	0.070
111	76	41	77	4.94	7.33	2.11	17	2.40	0.165	0.070
112	76	42	77	4.94	7.33	2.11	17	2.40	0.165	0.070
113	76	43	77	4.94	7.33	2.11	17	2.40	0.165	0.070
114	76	44	77	4.94	7.33	2.11	17	2.40	0.165	0.070
115	76	45	77	4.94	7.33	2.11	17	2.40	0.165	0.070
116	76	46	77	4.94	7.33	2.11	17	2.40	0.165	0.070
117	76	47	77	4.94	7.33	2.11	17	2.40	0.165	0.070
118	76	48	77	4.94	7.33	2.11	17	2.40	0.165	0.070
119	76	49	77	4.94	7.33	2.11	17	2.40	0.165	0.070
120	76	50	77	4.94	7.33	2.11	17	2.40	0.165	0.070
121	76	51	77	4.94	7.33	2.11	17	2.40	0.165	0.070
122	76	52	77	4.94	7.33	2.11	17	2.40	0.165	0.070
123	76	53	77	4.94	7.33	2.11	17	2.40	0.165	0.070
124	76	54	77	4.94	7.33	2.11	17	2.40	0.165	0.070
125	76	55	77	4.94	7.33	2.11	17	2.40	0.165	0.070
126	76	56	77	4.94	7.33	2.11	17	2.40	0.165	0.070
127	76	57	77	4.94	7.33	2.11	17	2.40	0.165	0.070
128	76	58	77	4.94	7.33	2.11	17	2.40	0.165	0.070
129	76	59	77	4.94	7.33	2.11	17	2.40	0.165	0.070
130	76	60	77	4.94	7.33	2.11	17	2.40	0.165	0.070
131	76	61	77	4.94	7.33	2.11	17	2.40	0.165	0.070
132	76	62	77	4.94	7.33	2.11	17	2.40	0.165	0.070
133	76	63	77	4.94	7.33	2.11	17	2.40	0.165	0.070
134	76	64	77	4.94	7.33	2.11	17	2.40	0.165	0.070
135	76	65	77	4.94	7.33	2.11	17	2.40	0.165	0.070
136	76	66	77	4.94	7.33	2.11	17	2.40	0.165	0.070
137	76	67	77	4.94	7.33	2.11	17	2.40	0.165	0.070
138	76	68	77	4.94	7.33	2.11	17	2.40	0.165	0.070
139	76	69	77	4.94	7.33	2.11	17	2.40	0.165	0.070
140	76	70	77	4.94	7.33	2.11	17	2.40	0.165	0.070
141	76	71	77	4.94	7.33	2.11	17	2.40	0.165	0.070
142	76	72	77	4.94	7.33	2.11	17	2.40	0.165	0.070
143	76	73	77	4.94	7.33	2.11	17	2.40	0.165	0.070
144	76	74	77	4.94	7.33	2.11	17	2.40	0.165	0.070
145	76	75	77	4.94	7.33	2.11	17	2.40	0.165	0.070
146	76	76	77	4.94	7.33	2.11	17	2.40	0.165	0.070
147	76	77	77	4.94	7.33	2.11	17	2.40	0.165	0.070
148	76	78	77	4.94	7.33	2.11	17	2.40	0.165	0.070
149	76	79	77	4.94	7.33	2.11	17	2.40	0.165	0.070
150	76	80	77	4.94	7.33	2.11	17	2.40	0.165	0.070
151	76	81	77	4.94	7.33	2.11	17	2.40	0.165	0.070
152	76	82	77	4.94	7.33	2.11	17	2.40	0.165	0.070
153	76	83	77	4.94	7.33	2.11	17	2.40	0.165	0.070
154	76	84	77	4.94	7.33	2.11	17	2.40	0.165	0.070
155	76	85	77	4.94	7.33	2.11	17	2.40	0.165	0.070
156	76	86	77	4.94	7.33	2.11	17	2.40	0.165	0.070
157	76	87	77	4.94	7.33	2.11	17	2.40	0.165	0.070
158	76	88	77	4.94	7.33	2.11	17	2.40	0.165	0.070
159	76	89	77	4.94	7.33	2.11	17	2.40	0.165	0.070
160	76	90	77	4.94	7.33	2.11	17	2.40	0.165	0.070
161	76	91	77	4.94	7.33	2.11	17	2.40	0.165	0.070
162	76	92	77	4.94	7.33	2.11	17	2.40	0.165	0.070
163	76	93	77	4.94	7.33	2.11	17	2.40	0.165	0.070
164	76	94	77	4.94	7.33	2.11	17	2.40	0.165	0.070
165	76	95	77	4.94	7.33	2.11	17	2.40	0.165	0.070
166	76	96	77	4.94	7.33	2.11	17	2.40	0.165	0.070
167	76	97	77	4.94	7.33	2.11	17	2.40	0.165	0.070
168	76	98	77	4.94	7.33	2.11	17	2.40	0.165	0.070
169	76	99	77	4.94	7.33	2.11	17	2.40	0.165	0.070
170	76	100	77	4.94	7.33	2.11	17	2.40	0.165	0.070
171	76	101	77	4.94	7.33	2.11	17	2.40	0.165	0.070
172	76	102	77	4.94	7.33	2.11	17	2.40	0.165	0.070
173	76	103	77	4.94	7.33	2.11	17	2.40	0.165	0.070
174	76	104	77	4.94	7.33	2.11	17	2.40	0.165	0.070
175	76	105	77	4.94	7.33	2.11	17	2.40	0.165	0.070
176	76	106	77	4.94	7.33	2.11	17	2.40	0.165	0.070
177	76	107	77	4.94	7.33	2.11	17	2.40	0.165	0.070
178	76	108	77	4.94	7.33	2.11	17	2.40	0.165	0.070
179	76	109	77	4.94	7.33	2.11	17	2.40	0.165	0.070
180	76	110	77	4.94	7.33	2.11	17	2.40	0.165	0.070
181	76	111	77	4.94	7.33	2.11	17	2.40	0.165	0.070
182	76	112	77	4.94	7.33	2.11	17	2.40	0.165	0.070
183	76	113	77	4.94	7.33	2.11	17	2.40	0.165	0.070
184	76	114	77	4.94	7.33	2.11	17	2.40	0.165	0.070
185	76	115	77	4.94	7.33	2.11	17	2.40	0.165	0.070
186	76	116	77	4.94	7.33	2.11	17	2.40	0.165	0.070
187	76	117	77	4.94	7.33	2.11	17	2.40	0.165	0.070
188	76	118	77	4.94	7.33	2.11	17	2.40	0.165	0.070
189	76	119	77	4.94	7.33	2.11	17	2.40	0.165	0.070
190	76	120	77	4.94	7.33	2.11	17	2.40	0.165	0.070
191	76	121	77	4.94	7.33	2.11	17	2.40	0.165	0.070
192	76	122	77	4.94	7.33	2.11	17	2.40	0.165	0.070
193	76	123	77	4.94	7.33	2.11	17	2.40	0.165	0.070
194	76	124	77	4.94	7.33	2.11	17	2.40	0.165	0.070
195	76	125	77	4.94	7.33	2.11	17	2.40	0.165	0.070
196	76	126	77	4.94	7.33	2.11	17	2.40	0.165	0.070
197	76	127	77	4.94	7.33	2.11	17	2.40	0.165	0.070
198	76	128	77	4.94	7.33	2.11	17	2.40	0.165	0.070
199	76	129	77	4.94	7.33	2.11	17	2.40	0.165	0.070
200	76	130	77	4.94	7.33	2.11	17	2.40	0.165	0.070
201	76	131	77	4.94	7.33	2.11	17	2.40	0.165	0.070
202	76	132	77	4.94	7.33	2.11	17	2.40	0.165	0.070
203	76	133	77	4.94	7.33	2.11	17	2.40	0.165	0.07

BLOOD PLASMA DATA										
OBS	ANIMAL	PERIOD	TRT	PP	PCA	PMC	PK	PNA	PZN	PCU
168	168	1	1	.	.	.	.	.	.	.
168	168	1	1	.	.	.	.	.	.	.
168	168	1	1	.	.	.	.	.	.	.
168	168	1	1	5.87	9.78	2.12	21	333	0.094	0.052
168	168	1	1	4.13	11.91	2.23	23	333	0.106	0.073
168	168	1	1	23.31	11.42	2.23	23	2242	0.118	0.068
168	168	1	1	2.60	11.34	2.23	19	2243	0.091	0.061
168	168	1	1	4.32	10.06	2.23	19	2258	0.173	0.072
168	168	1	1	4.30	8.09	2.19	23	2243	0.202	0.091
168	168	1	1	3.31	8.16	2.23	19	215	0.274	0.098
169	169	1	1	1.93	10.25	2.00	16	313	0.144	0.057
169	169	1	1	1.87	11.29	1.86	17	236	0.098	0.058
169	169	1	1	2.93	11.27	2.36	19	347	0.145	0.086
169	169	1	1	4.00	12.18	2.22	20	237	0.118	0.059
169	169	1	1	1.78	12.43	2.22	14	221	0.087	0.082
169	169	1	1	3.90	9.84	2.22	16	221	0.175	0.065
169	169	1	1	4.64	8.32	1.93	23	239	0.219	0.074
169	169	1	1	4.82	8.78	1.98	23	260	0.227	0.111
170	170	1	1	1.79	10.58	2.07	15	320	0.160	0.071
170	170	1	1	2.02	11.76	1.89	15	297	0.102	0.079
170	170	1	1	4.17	10.14	2.22	17	350	0.192	0.083
170	170	1	1	2.53	12.81	2.22	20	257	0.113	0.053
170	170	1	1	3.72	10.40	2.23	19	233	0.116	0.094
170	170	1	1	1.54	10.67	1.96	12	241	0.103	0.104
170	170	1	1	3.62	9.43	1.93	15	224	0.173	0.099
170	170	1	1	3.32	8.12	1.97	.	.	0.203	0.080
170	170	1	1	3.72	7.79	2.10	.	.	0.221	0.114
171	171	1	1	4.27	10.20	2.37	15	314	0.136	0.087
171	171	1	1	2.64	12.73	2.40	16	304	0.178	0.091
171	171	1	1	.	.	.	.	.	.	.
171	171	1	1	3.86	10.88	2.56	19	229	0.168	0.097
171	171	1	1	2.46	10.50	2.03	14	215	0.088	0.102
171	171	1	1	5.85	9.08	2.17	17	220	0.168	0.109
171	171	1	1	5.99	7.86	1.92	.	.	0.195	0.087
171	171	1	1	4.13	8.52	2.07	21	245	0.205	0.099
172	172	1	1	.	.	.	.	.	.	.
172	172	1	1	.	.	.	.	.	.	.
172	172	1	1	7.55	11.14	2.46	15	342	0.114	0.069
172	172	1	1	4.53	12.94	2.36	19	370	0.058	0.054
172	172	1	1	3.59	11.60	2.26	21	243	0.056	0.078
172	172	1	1	1.78	10.73	2.24	17	230	0.097	0.086
172	172	1	1	2.51	11.06	2.42	18	235	0.118	0.054
172	172	1	1	4.09	8.80	2.07	17	250	0.213	0.076
173	173	1	1	.	.	.	.	.	.	.
173	173	1	1	4.13	9.72	2.03	16	283	0.144	0.063
173	173	1	1	2.33	11.19	2.03	.	276	0.168	0.057
173	173	1	1	.	.	.	.	.	.	.
173	173	1	1	4.13	11.63	2.16	17	323	0.093	0.061
173	173	1	1	3.59	11.41	2.79	22	223	0.111	0.099
173	173	1	1	1.51	11.02	1.65	12	230	0.089	0.104
173	173	1	1	3.34	9.63	1.99	17	203	0.168	0.165
173	173	1	1	4.64	8.21	2.03	.	.	0.191	0.106
174	174	1	1	.	.	.	.	.	.	.
174	174	1	1	.	.	.	.	.	.	.
174	174	1	1	.	.	.	.	.	.	.
174	174	1	1	2.93	11.95	2.26	23	331	0.148	0.056
174	174	1	1	4.00	10.67	2.70	22	238	0.111	0.092
174	174	1	1	2.46	10.65	2.12	17	227	0.098	0.091
174	174	1	1	5.16	9.15	2.02	17	.	0.188	0.117
174	174	1	1	4.23	8.61	2.14	.	210	0.212	0.109
174	174	1	1	3.31	8.80	2.14	25	248	0.213	0.140
176	176	1	1	.	.	.	.	.	.	.
176	176	1	1	3.99	9.05	2.01	20	293	0.137	0.079
176	176	1	1	2.33	10.46	1.94	14	329	0.081	0.064
176	176	1	1	.	.	.	.	.	.	.
176	176	1	1	3.60	11.89	2.46	24	399	0.117	0.081

BLOOD PLASMA DATA										
OBS	ANIMAL	PERIOD	TRT	PP	PCA	PMG	PK	PNA	PZN	PCU
76	276	6	N	3.31	11.34	2.73	27.00	221	0.093	0.062
77	276	7	N	4.32	10.23	2.00	19.00	2210	0.072	0.041
78	276	8	N	3.37	8.43	2.01	15.00	2216	0.170	0.117
79	276	9	N	4.30	7.59	1.46	11.00	2217	0.208	0.096
80	276	10	N	3.76	7.98	1.76	14.00	224	0.251	0.109
81	277	1	N	2.48	10.34	2.18	17.00	2281	0.129	0.063
82	277	2	N	2.33	12.28	2.00	17.00	2272	0.110	0.048
83	277	3	N	4.44	10.39	1.45	21.00	2251	0.109	0.081
84	277	4	N	4.00	11.77	2.00	21.00	2270	0.186	0.043
85	277	5	N	3.86	13.34	2.49	21.00	2213	0.080	0.043
86	277	6	N	4.66	9.84	2.00	12.00	2234	0.081	0.086
87	277	7	N	4.18	8.33	2.13	13.00	2233	0.170	0.108
88	277	8	N	4.77	7.47	2.00	13.00	2200	0.177	0.084
89	277	9	N	3.86	7.39	1.42	22.00	2244	0.176	0.099
90	277	10	N	...	...	...	...	...	...	...
91	278	1	N	...	...	...	...	...	...	...
92	278	2	N	...	...	...	...	...	...	...
93	278	3	N	...	...	...	...	...	...	...
94	278	4	N	...	...	...	...	...	...	...
95	278	5	N	4.40	11.62	2.40	...	...	0.074	0.052
96	278	6	N	3.86	10.46	2.86	26.00	2236	0.093	0.084
97	278	7	N	3.73	8.91	2.11	13.00	2206	0.102	0.084
98	278	8	N	3.02	8.19	2.38	17.00	2208	0.193	0.098
99	278	9	N	4.91	8.15	2.33	14.00	2204	0.212	0.081
100	278	10	N	...	...	...	...	...	...	...
101	279	1	N	2.76	10.31	2.09	18.00	2296	0.199	0.086
102	279	2	N	1.70	11.20	2.04	17.00	2296	0.063	0.077
103	279	3	N	...	...	...	...	...	...	...
104	279	4	N	2.46	11.64	2.24	29.00	...	0.191	0.053
105	279	5	N	4.41	12.63	2.85	18.00	227	0.108	0.091
106	279	6	N	6.71	9.48	1.77	18.00	2238	0.083	0.078
107	279	7	N	3.30	8.37	2.03	15.00	2217	0.199	0.113
108	279	8	N	3.18	8.10	2.33	20.00	2231	0.242	0.091
109	279	9	N	4.27	7.96	1.76	16.00	2235	0.237	0.105
110	279	10	N	...	...	...	...	...	...	...
111	280	1	N	3.17	9.79	1.96	22.00	2216	0.122	0.063
112	280	2	N	3.26	12.00	2.13	19.00	222	0.083	0.077
113	280	3	N	...	...	...	...	...	...	...
114	280	4	N	4.13	12.08	2.41	23.00	407	0.083	0.059
115	280	5	N	4.13	10.83	2.71	21.00	2236	0.093	0.068
116	280	6	N	3.73	10.13	2.32	16.00	2237	0.094	0.066
117	280	7	N	3.99	8.06	2.01	20.00	2247	0.199	0.068
118	280	8	N	3.86	8.50	2.28	...	2228	0.250	0.084
119	280	9	N	...	...	...	...	...	...	...
120	280	10	N	...	...	...	...	...	...	...
121	281	1	N	4.41	10.37	1.99	17.00	2212	0.120	0.090
122	281	2	N	1.33	11.49	2.34	15.00	2264	0.119	0.067
123	281	3	N	...	...	...	...	...	...	...
124	281	4	N	3.33	11.49	2.36	...	...	0.131	0.070
125	281	5	N	2.48	12.12	2.96	18.00	2233	0.089	0.087
126	281	6	N	3.07	9.21	2.33	23.00	2239	0.122	0.110
127	281	7	N	3.93	7.94	1.98	17.00	2234	0.205	0.126
128	281	8	N	4.36	7.86	1.83	0.13	2236	0.218	0.092
129	281	9	N	3.89	8.24	1.89	17.00	2242	0.217	0.107
130	281	10	N	...	...	...	...	...	...	...
131	282	1	N	3.38	9.72	2.37	19.00	2217	0.134	0.078
132	282	2	N	3.38	11.61	2.32	18.00	2285	0.111	0.069
133	282	3	N	...	...	...	...	...	...	...
134	282	4	N	3.33	11.19	2.47	29.00	...	0.111	0.064
135	282	5	N	4.97	11.63	2.97	24.00	2233	0.087	0.072
136	282	6	N	3.48	9.44	1.16	13.00	2200	0.093	0.105
137	282	7	N	3.33	8.33	2.33	18.00	2234	0.093	0.085
138	282	8	N	4.36	7.48	2.77	...	220	0.240	0.087
139	282	9	N	4.41	8.39	2.44	...	220	0.208	0.085
140	282	10	N	...	...	...	...	...	...	...

OBS	ANIMAL	PERIOD	TRT	WHOLE BLOOD DATA						
				WBP	WBCA	WBMG	WBSK	WBSNA	WBSZN	WBCU
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	11.07	9.85	2.30	48	224	0.576	0.071
1	168	1	1	14.62	7.05	1.95	57	222	0.420	0.074
1	168	1	1	13.18	8.50	2.25	43	224	0.720	0.081
1	168	1	1	8.93	6.90	2.10	52	227	0.691	0.076
1	168	1	1	11.88	8.05	2.10	46	225	0.403	0.090
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	11.31	7.80	2.15	46	249	0.440	0.068
1	168	1	1	11.13	7.22	1.75	38	263	0.470	0.076
1	168	1	1	.	.	.	.	.	.	.
1	168	1	1	10.04	8.35	2.05	55	240	0.384	0.081
1	168	1	1	11.74	7.25	1.90	44	266	0.490	0.078
1	168	1	1	23.10	8.90	2.15	43	215	0.740	0.044
1	168	1	1	9.02	6.35	2.00	51	232	0.490	0.080
1	168	1	1	10.22	7.05	1.85	58	237	0.474	0.083
1	170	1	1	.	.	.	.	.	.	.
1	170	1	1	9.71	7.50	1.95	35	259	0.420	0.088
1	170	1	1	.	.	.	.	.	.	.
1	170	1	1	10.80	7.65	2.00	46	223	0.417	0.075
1	170	1	1	10.30	7.10	1.80	53	222	0.640	0.082
1	170	1	1	17.28	7.75	2.05	44	222	0.650	0.081
1	170	1	1	12.95	6.50	2.25	55	226	0.844	0.082
1	170	1	1	9.46	7.15	2.00	54	251	0.533	0.085
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	11.92	7.85	2.00	28	249	0.300	0.080
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	13.26	8.10	1.95	54	220	0.606	0.088
1	171	1	1	9.13	6.75	1.75	64	223	0.320	0.088
1	171	1	1	12.63	7.40	2.05	54	223	0.570	0.088
1	171	1	1	9.91	6.90	2.05	51	234	0.510	0.081
1	171	1	1	10.13	7.70	1.90	57	263	0.576	0.078
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	10.63	9.25	2.20	48	226	0.565	0.077
1	171	1	1	7.80	7.30	1.80	53	226	0.390	0.075
1	171	1	1	17.76	9.50	2.20	40	223	0.540	0.076
1	171	1	1	10.41	7.70	2.10	41	258	0.596	0.076
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	9.84	7.65	2.10	35	246	0.280	0.076
1	171	1	1	10.65	7.20	1.90	42	268	0.300	0.078
1	171	1	1	.	.	.	.	.	.	.
1	171	1	1	8.57	9.35	2.20	36	268	0.673	0.090
1	171	1	1	9.15	7.60	1.50	36	235	0.520	0.076
1	171	1	1	19.78	9.65	1.90	44	244	0.930	0.078
1	171	1	1	11.33	7.15	2.10	54	253	0.472	0.093
1	174	1	1	.	.	.	.	.	.	.
1	174	1	1	.	.	.	.	.	.	.
1	174	1	1	12.21	8.25	1.85	45	242	0.770	0.081
1	174	1	1	8.93	7.80	2.05	32	251	0.774	0.076
1	174	1	1	.	.	.	.	.	.	.
1	174	1	1	21.70	8.05	1.95	44	239	0.720	0.047
1	174	1	1	9.51	6.75	2.20	43	219	0.524	0.087
1	174	1	1	11.70	8.65	2.05	46	275	0.870	0.095
1	176	1	1	.	.	.	.	.	.	.
1	176	1	1	9.59	7.35	2.00	53	222	0.270	0.077
1	176	1	1	.	.	.	.	.	.	.
1	176	1	1	.	.	.	.	.	.	.

OBS	ANIMAL	PERIOD	TRT	WHOLE BLOOD DATA						
				WBP	WBCA	WBMC	WBK	WBNA	WBZN	WBCU
76	276	6	1	10.19	7.50	1.80	45	237	0.427	0.079
77	276	7	1	12.25	7.75	1.75	45	245	0.640	0.088
78	276	8	1	11.34	6.35	2.00	46	240	0.660	0.097
79	276	9	1	9.02	7.30	1.75	52	263	0.610	0.092
80	276	10	1	.	.	.	.	.	.	.
81	277	1	2	.	.	.	.	.	.	.
82	277	2	2	.	.	.	.	.	.	.
83	277	3	2	13.36	9.65	2.25	38	233	0.760	0.070
84	277	4	2	.	.	.	.	.	.	.
85	277	5	2	.	.	.	.	.	.	.
86	277	6	2	11.83	8.90	2.40	32	247	0.690	0.065
87	277	7	2	14.75	7.35	1.90	46	242	0.700	0.085
88	277	8	2	13.30	7.35	2.00	49	249	0.690	0.080
89	277	9	2	9.87	6.50	1.50	39	273	0.447	0.090
90	277	10	2	9.04	7.70	2.00	35	293	0.656	0.163
91	278	1	1	.	.	.	.	.	.	.
92	278	2	1	.	.	.	.	.	.	.
93	278	3	1	.	.	.	.	.	.	.
94	278	4	1	.	.	.	.	.	.	.
95	278	5	1	.	.	.	.	.	.	.
96	278	6	1	11.12	8.05	2.00	46	235	0.505	0.078
97	278	7	1	15.21	8.80	2.00	42	242	0.500	0.081
98	278	8	1	20.76	8.40	2.00	40	243	0.600	0.079
99	278	9	1	11.61	6.45	2.00	44	227	0.932	0.083
100	278	10	1	.	.	.	.	.	.	.
101	279	1	1	.	.	.	.	.	.	.
102	279	2	1	.	.	.	.	.	.	.
103	279	3	1	11.02	8.60	2.30	39	245	0.450	0.064
104	279	4	1	9.55	8.00	1.90	35	265	0.360	0.076
105	279	5	1	.	.	.	.	.	.	.
106	279	6	1	11.74	9.60	2.15	40	233	0.700	0.077
107	279	7	1	16.86	6.80	1.80	42	231	0.400	0.073
108	279	8	1	21.88	6.95	1.90	48	225	0.760	0.051
109	279	9	1	12.34	7.70	2.00	38	245	0.470	0.073
110	279	10	1	10.80	7.65	2.00	46	288	0.556	0.083
111	280	1	1	.	.	.	.	.	.	.
112	280	2	1	.	.	.	.	.	.	.
113	280	3	1	14.43	7.95	1.95	43	263	0.330	0.071
114	280	4	1	.	.	.	.	.	.	.
115	280	5	1	.	.	.	.	.	.	.
116	280	6	1	10.27	8.40	2.10	41	251	0.523	0.083
117	280	7	1	11.23	7.10	2.00	43	231	0.750	0.083
118	280	8	1	10.74	6.40	2.00	43	231	0.900	0.075
119	280	9	1	11.70	6.15	2.00	40	247	0.634	0.076
120	280	10	1	.	.	.	.	.	.	.
121	281	1	2	.	.	.	.	.	.	.
122	281	2	2	.	.	.	.	.	.	.
123	281	3	2	12.13	7.75	2.25	28	258	0.390	0.074
124	281	4	2	.	.	.	.	.	.	.
125	281	5	2	.	.	.	.	.	.	.
126	281	6	2	10.13	6.60	1.90	40	226	0.553	0.078
127	281	7	2	13.31	6.05	1.85	47	279	0.700	0.080
128	281	8	2	18.38	6.70	1.90	39	268	0.400	0.078
129	281	9	2	8.26	6.70	2.00	37	243	0.503	0.083
130	281	10	2	9.24	6.65	1.95	39	233	0.856	0.090
131	282	1	1	.	.	.	.	.	.	.
132	282	2	1	.	.	.	.	.	.	.
133	282	3	1	15.57	8.75	2.05	40	258	0.340	0.069
134	282	4	1	.	.	.	.	.	.	.
135	282	5	1	.	.	.	.	.	.	.
136	282	6	1	12.54	8.70	2.10	48	256	0.400	0.073
137	282	7	1	15.08	6.75	2.00	33	235	0.660	0.076
138	282	8	1	14.19	6.20	2.00	33	279	0.450	0.079
139	282	9	1	15.27	6.80	2.00	35	256	0.551	0.092
140	282	10	1	11.80	6.90	2.00	35	247	0.652	0.072

HEMOGLOBIN AND HEMATOCRIT DATA

OBS	ANIMAL	PERIOD	TRT	Hb	PCV
1	168	1	1	10.85	31.5
2	168		1		
3	168		1		
4	168	4	1	11.00	28.5
5	168	5	1	10.12	29.5
6	168	6	1	13.68	33.5
7	168	7	1	13.62	38.0
8	168	8	1	13.61	37.5
9	168	9	1	14.41	36.5
10	168	10	1	10.48	33.5
11	169	11	1	10.28	31.0
12	169	12	1	12.40	31.5
13	169	13	1		
14	169	14	1	13.16	31.5
15	169	15	1	13.44	36.5
16	169	16	1	18.40	40.5
17	169	17	1	15.33	44.0
18	169	18	1	16.32	42.0
19	169	19	1	16.23	41.5
20	169	20	1	14.34	37.0
21	170	21	1		
22	170	22	1	13.60	32.1
23	170	23	1		
24	170	24	1	14.51	34.5
25	170	25	1	11.13	29.0
26	170	26	1	13.56	37.5
27	170	27	1	15.78	44.0
28	170	28	1	16.82	41.5
29	170	29	1	16.72	42.0
30	170	30	1	14.56	41.5
31	171	31	1		
32	171	32	1	13.51	31.5
33	171	33	1		
34	171	34	1	11.95	34.5
35	171	35	1	11.00	31.0
36	171	36	1	17.82	38.5
37	171	37	1	14.64	40.0
38	171	38	1	15.16	37.5
39	171	39	1	14.85	36.5
40	171	40	1	12.84	35.5
41	172	41	1	12.16	28.5
42	172	42	1		
43	172	43	1		
44	172	44	1	10.61	33.0
45	172	45	1	10.00	33.5
46	172	46	1	13.50	32.0
47	172	47	1	11.85	34.0
48	172	48	1	13.93	36.5
49	172	49	1	13.23	35.0
50	172	50	1		
51	173	51	1		
52	173	52	1	10.89	27.5
53	173	53	1		
54	173	54	1	11.45	29.0
55	173	55	1	11.78	32.0
56	173	56	1	13.12	38.5
57	173	57	1	13.80	39.0
58	173	58	1	14.73	43.5
59	173	59	1	13.44	35.5
60	173	60	1		
61	174	61	1	11.95	35.0
62	174	62	1		
63	174	63	1		
64	174	64	1	11.95	33.5
65	174	65	1	10.72	32.5
66	174	66	1	13.11	37.5
67	174	67	1	13.62	40.0
68	174	68	1	13.16	40.0
69	174	69	1	13.07	38.5
70	174	70	1	13.76	40.0
71	176	71	1	10.85	31.5
72	176	72	1	10.40	28.5
73	176	73	1		
74	176	74	1		
75	176	75	1	10.27	30.5

HEMOGLOBIN AND HEMATOCRIT DATA

QBS	ANIMAL	PERIOD	TRT	H9	PCV
76	276	6	N	15.57	33.40
77	276	7	N	14.01	33.00
78	276	8	N	13.84	33.00
79	276	9	N	13.07	33.00
80	276	10	N	13.87	33.00
81	277	11	N	11.04	28.00
82	277	12	N	10.73	33.00
83	277	13	N	10.47	33.00
84	277	14	N	10.96	33.00
85	277	15	N	12.01	33.00
86	277	16	N	13.37	33.00
87	277	17	N	14.41	33.00
88	277	18	N	13.36	33.00
89	277	19	N	10.35	33.00
90	278	20	N	10.47	33.00
91	278	21	N	11.78	33.00
92	278	22	N	12.02	33.00
93	278	23	N	13.24	33.00
94	278	24	N	13.84	33.00
95	278	25	N	13.84	33.00
96	278	26	N	13.84	33.00
97	278	27	N	13.84	33.00
98	278	28	N	13.84	33.00
99	278	29	N	13.84	33.00
100	278	30	N	13.84	33.00
101	279	31	N	10.25	27.00
102	279	32	N	10.71	33.00
103	279	33	N	10.00	33.00
104	279	34	N	16.27	33.00
105	279	35	N	12.87	33.00
106	279	36	N	13.16	33.00
107	279	37	N	13.84	33.00
108	279	38	N	13.84	33.00
109	279	39	N	13.84	33.00
110	279	40	N	13.84	33.00
111	280	41	N	12.60	31.00
112	280	42	N	12.08	33.00
113	280	43	N	14.69	33.00
114	280	44	N	14.01	33.00
115	280	45	N	14.51	33.00
116	280	46	N	14.64	33.00
117	280	47	N	13.55	33.00
118	280	48	N	13.80	33.00
119	280	49	N	13.14	33.00
120	280	50	N	13.12	33.00
121	281	51	N	13.44	33.00
122	281	52	N	13.44	33.00
123	281	53	N	13.44	33.00
124	281	54	N	13.44	33.00
125	281	55	N	13.44	33.00
126	281	56	N	13.44	33.00
127	281	57	N	13.44	33.00
128	281	58	N	13.44	33.00
129	281	59	N	13.44	33.00
130	281	60	N	13.44	33.00
131	282	61	N	12.02	33.00
132	282	62	N	12.02	33.00
133	282	63	N	12.02	33.00
134	282	64	N	12.02	33.00
135	282	65	N	12.02	33.00
136	282	66	N	12.02	33.00
137	282	67	N	12.02	33.00
138	282	68	N	12.02	33.00
139	282	69	N	12.02	33.00
140	282	70	N	12.02	33.00

Ovarian Volume Palpation Data Codes

Animal = Cow number

Period 1 = October 14, 1983
2 = November 10, 1983
3 = December 16, 1983
4 = January 16, 1984
5 = February 17, 1984
6 = March 16, 1984

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

R-Ovary = Right ovary volume (mm^3)

L-Ovary = Left ovary volume (mm^3)

Total = Right ovary volume + left ovary volume (mm^3)

OVARIAN VOLUME PALPATION DATA

OBS	ANIMAL	PERIOD	TRT	R_OVARY	L_OVARY	TOTAL
168	1	1	1	1800	1200	3000
169	1	1	1	1680	2688	4368
170	1	1	1	3360	1680	5040
171	1	1	1	1920	1600	3520
172	1	1	1	1400	2200	3600
173	1	1	1	1680	2688	4368
174	1	1	1	1920	1200	3120
175	1	1	1	3360	1600	4960
176	1	1	1	1200	1440	2640
177	1	1	1	1920	1680	3600
178	1	1	1	3024	1200	4224
179	1	1	1	1920	1200	3120
180	1	1	1	7680	1440	9120
181	1	1	1	1440	1680	3120
182	1	1	1	1680	4444	6124
183	1	1	1	4032	7920	11952
184	1	1	1	2688	5760	8448
185	1	1	1	1680	4480	6160
186	1	1	1	1680	1440	3120
187	1	1	1	4480	2688	7168
188	1	1	1	1680	1200	2880
189	1	1	1	5040	6336	11376
190	1	1	1	3024	22688	25712
191	1	1	1	1680	22688	24368
192	1	1	1	3024	4480	7504
193	1	1	1	1200	4032	5232
194	1	1	1	15840	3024	19864
195	1	1	1	3360	1680	5040
196	1	1	1	6400	1680	8080
197	1	1	1	6400	3540	9940
198	1	1	1	4032	1680	5712
199	1	1	1	2688	1440	4128
200	1	1	1	1680	3456	5136
201	1	1	1	4928	2688	7616
202	1	1	1	2304	5040	7344
203	1	1	1	3024	4928	7952
204	1	1	1	3360	6336	9696
205	1	1	1	3024	1680	4704
206	1	1	1	3660	3528	7188
207	1	1	1	1680	1200	2880
208	1	1	1	3024	13200	16224
209	1	1	1	6048	2688	8736
210	1	1	1	5760	4032	9792
211	1	1	1	11440	7040	18480
212	1	1	1	4032	13728	17760
213	1	1	1	13440	3024	16464
214	1	1	1	4480	4536	9016
215	1	1	1	3360	3024	6384
216	1	1	1	5376	7040	12416
217	1	1	1	16016	5544	21560
218	1	1	1	3696	7856	11552
219	1	1	1	456	3780	4236
220	1	1	1	7680	2688	10368
221	1	1	1	6336	3456	9792
222	1	1	1	1600	8640	10240
223	1	1	1	8640	4224	12864
224	1	1	1	3024	13728	16752
225	1	1	1	.	.	.
226	1	1	1	5544	6048	11592
227	1	1	1	6048	8640	14688
228	1	1	1	6048	12320	18368
229	1	1	1	4480	3024	7504
230	1	1	1	4224	7680	11904
231	1	1	1	5544	11440	16984
232	1	1	1	.	.	.
233	1	1	1	.	.	.
234	1	1	1	.	.	.
235	1	1	1	8800	3024	11824

OVARIAN VOLUME PALPATION DATA						
Q3S	ANIMAL	PERIOD	TRT	R_OVARY	L_OVARY	TOTAL
76	173	6	1	4480	10816	15296
77	174	6				
78	276	6				
79	277	6				
80	278	6		4032	2688	6720
81	279	6		7680	2280	9960
82	280	6				
83	281	6				
84	282	6				

Third Metacarpal (McIII) Data Codes

Cow_No = Cow number

Leg L = Left leg

R = Right leg

Trt 1 = Low phosphorus (.12% P, DMB)

2 = High phosphorus (.20% P, DMB)

Spine = Photon absorption spine scan bone mineral density
($\text{g}\cdot\text{cm}^{-2}$), whole bone

PABMC = Photon absorption bone mineral content (g), whole bone

BMCW = Bone mineral content/width ($\text{g}\cdot\text{cm}^{-1}$), whole bone

CD = Corpus density ($\text{mg}\cdot\text{cm}^{-3}$), whole bone

CBI = Cortical bone index (%)

MLWT = Medial lateral wall thickness (mm)

BOMB = Breadth of mineralized bone (cm)

BS = Breaking strength ($\text{kg}\cdot\text{cm}^{-2}$)

BL = Breaking load (kg)

MIE = Moment of inertia-ellipse (cm^4)

E = Young's modulus ($\text{kg}\cdot\text{cm}^{-2}$)

STRAIN = Strain (change in length per unit length)

CAI_1 = Circular area index-break section (mm^2)

EAI_1 = Elliptical area index-break section (mm^2)

XRI_1 = Radiographic area index-break section (mm^2)

PAI_1 = Planimeter area index-break section (mm^2)

BMC_1 = Bone mineral content-break section (g)

ASH_1 = Bone ash-break section (%)

P_1 = Phosphorus as % of bone ash-break section

CA_1 = Calcium as % of bone ash-break section

MG_1 = Magnesium as % of bone ash-break section

PBMC_1 = Photon absorption bone mineral content-break section (g)

PHOTON_1 = Photon absorption bone mineral density-break section
($\text{g}\cdot\text{cm}^{-2}$)

US-1 = Ultrasound-break section ($\text{m}\cdot\text{s}^{-1}$)

RBAE_1 = Radiographic bone aluminum equivalence-break section (mm,
Al)

All variables with _2 designation are for the section 1.0 cm proximal to the break section. All variable units are as described for the break section.

THIRD METACARPAL (McIII) MECHANICAL PROPERTIES DATA

OBS	COW_NO	LEG	TRT	BS	BL	MIE	E	STRAIN
1	168	L	1	1841	920	1.80	23513	0.0782971
2	168	R	1	1983	1024	1.90	25951	0.0764132
3	169	L	1	2119	1390	2.67	26478	0.0800287
4	169	R	1	1753	1230	2.89	21638	0.0810149
5	170	L	1	1976	1340	2.77	23303	0.0856542
6	170	R	1	2184	1424	2.83	29008	0.0752896
7	171	L	1	1887	1270	2.78	24576	0.0767822
8	171	R	1	1873	1216	2.63	24877	0.0752904
9	172	L	1	1988	1190	2.31	26150	0.0760229
10	172	R	1	2035	1120	2.06	31086	0.0654636
11	173	L	1	1813	1184	2.57	22176	0.0817551
12	173	R	1	1706	1156	2.51	26338	0.0647733
13	174	L	1	1818	1070	2.38	24252	0.0749629
14	174	R	1	2009	970	1.94	28588	0.0702742
15	276	L	1	2106	1394	2.62	32497	0.0648060
16	276	R	1	2190	1480	2.70	35826	0.0611288
17	277	L	1	2308	1640	2.85	30961	0.0745454
18	277	R	1	1934	1573	2.48	22056	0.0876859
19	278	L	1	2031	1353	2.70	28771	0.0705919
20	278	R	1	2014	1304	2.60	24178	0.0832989
21	279	L	1	2029	1298	2.82	27780	0.0730382
22	279	R	1	2111	1300	2.39	31097	0.0678844
23	280	L	1	2138	1246	2.23	23249	0.0919609
24	280	R	1	2108	1216	2.18	24324	0.0866634
25	281	L	1	1978	1264	2.48	31077	0.0636484
26	281	R	1	1805	1270	2.86	25370	0.0711470
27	282	L	1	2053	1250	2.32	25906	0.0792481
28	282	R	1	2101	1270	2.33	29320	0.0716576

THIRD METACARPAL (McIII) BREAK SECTION AREA INDICES DATA

OBS	COW_NO	LEG	TRT	CAI_1	EAI_1	XRI_1	PAI_1
1	168	L	1	890.47	558.95	890.69	488.81
2	168	R	1	916.88	569.11	912.13	475.84
3	169	L	1	853.73	624.10	915.04	518.92
4	169	R	1	1040.76	726.12	1053.44	562.76
5	170	L	1	888.72	627.55	900.24	571.63
6	170	R	1	1091.43	734.34	1117.40	563.09
7	171	L	1	887.68	571.96	935.50	577.53
8	171	R	1	894.72	654.11	947.89	525.74
9	172	L	1	970.27	648.97	954.55	528.52
10	172	R	1	900.55	624.11	1007.63	516.04
11	173	L	1	741.22	650.83	933.10	488.41
12	173	R	1	939.12	635.96	965.79	523.82
13	174	L	1	975.94	643.44	953.91	520.26
14	174	R	1	846.88	561.40	915.26	455.33
15	276	L	N	1055.66	690.82	1090.44	577.16
16	276	R	N	1071.56	701.61	1145.25	580.72
17	277	L	N	1188.48	762.47	1273.78	619.87
18	277	R	N	1271.81	836.25	1295.68	660.13
19	278	L	N	977.82	678.92	954.85	565.21
20	278	R	N	985.05	678.91	976.08	559.45
21	279	L	N	1051.20	696.38	1042.80	577.45
22	279	R	N	990.38	619.66	1044.00	536.32
23	280	L	N	996.18	639.11	1007.17	557.65
24	280	R	N	979.59	633.52	1103.79	542.06
25	281	L	N	1080.72	672.38	997.20	577.00
26	281	R	N	1015.75	705.66	1019.04	536.86
27	282	L	N	999.16	654.30	986.74	543.88
28	282	R	N	1009.56	632.11	1032.66	548.26

THIRD METACARPAL (McIII) BREAK SECTION CHEMICAL ANALYSIS DATA

OBS	COW_NO	LEG	TRT	IMC_1	ASH_1	P_1	CA_1	MG_1
1	168	L	1	10.25	67.10	16.5	36.2	0.51
2	168	R	1	10.97	67.80	16.1	36.9	0.51
3	169	L	1	12.36	67.35	16.2	37.2	0.51
4	169	R	1	12.29	68.20	16.3	36.9	0.50
5	170	L	1	12.42	67.60	16.4	37.4	0.57
6	170	R	1	12.70	67.40	16.0	37.4	0.57
7	171	L	1	11.23	66.90	15.9	37.1	0.51
8	171	R	1	11.43	67.70	15.9	37.7	0.53
9	172	L	1	10.55	67.00	15.9	37.6	0.55
10	172	R	1	10.61	66.50	15.8	37.4	0.53
11	173	L	1	11.41	66.80	15.9	38.3	0.51
12	173	R	1	11.38	66.90	16.0	38.2	0.53
13	174	L	1	9.80	66.40	16.4	36.9	0.50
14	174	R	1	9.43	66.90	15.6	36.2	0.52
15	276	L	1	12.76	68.80	17.2	38.1	0.49
16	276	R	1	12.96	68.90	17.5	38.2	0.51
17	277	L	1	13.95	68.40	16.7	38.8	0.52
18	277	R	1	14.01	67.90	16.0	36.2	0.53
19	278	L	1	11.76	67.20	16.2	38.1	0.60
20	278	R	1	11.64	66.70	15.9	37.4	0.59
21	279	L	1	12.40	67.90	16.2	36.3	0.54
22	279	R	1	12.02	69.30	16.5	37.1	0.53
23	280	L	1	12.52	67.80	15.9	37.4	0.52
24	280	R	1	12.33	67.60	15.8	36.9	0.51
25	281	L	1	12.70	68.80	16.5	36.6	0.55
26	281	R	1	12.53	68.40	16.4	36.9	0.55
27	282	L	1	12.41	67.70	16.5	37.0	0.55
28	282	R	1	12.61	68.00	16.3	36.5	0.54

THIRD METACARPAL (McIII) BREAK SECTION NON-INVASIVE TECHNIQUES DATA

OBS	COW_NO	LEG	TRT	PHMC_1	PHOTON_1	US_1	RBAE_1
1	168	L	1	11.5	1.325	3263.6	12.82
2	168	R	1	11.3	1.322	3326.7	13.42
3	169	L	1	12.9	1.427	3272.6	14.31
4	169	R	1	12.9	1.400	3264.1	14.10
5	170	L	1	12.5	1.445	3267.7	14.62
6	170	R	1	13.0	1.466	3393.3	14.92
7	171	L	1	12.1	1.422	3215.8	13.81
8	171	R	1	12.7	1.469	3242.5	13.65
9	172	L	1	11.8	1.414	3234.7	13.21
10	172	R	1	11.5	1.339	3208.5	13.31
11	173	L	1	13.2	1.521	3122.9	13.76
12	173	R	1	12.9	1.476	3123.1	13.43
13	174	L	1	10.6	1.277	3270.8	12.65
14	174	R	1	10.3	1.204	3316.2	12.32
15	276	L	N	14.3	1.525	3364.5	15.21
16	276	R	N	14.2	1.591	3405.7	14.88
17	277	L	N	16.2	1.760	3363.7	16.24
18	277	R	N	16.3	1.759	3513.4	15.85
19	278	L	N	13.3	1.543	3260.5	14.38
20	278	R	N	12.9	1.455	3275.6	14.43
21	279	L	N	13.9	1.603	3394.1	14.66
22	279	R	N	13.4	1.547	3246.8	14.62
23	280	L	N	13.6	1.546	3196.8	14.71
24	280	R	N	13.0	1.471	3201.5	14.67
25	281	L	N	13.3	1.480	3370.8	15.36
26	281	R	N	13.1	1.457	3350.0	14.97
27	282	L	N	13.3	1.492	3405.2	14.66
28	282	R	N	13.7	1.472	3413.7	14.91

THIRD METACARPAL (McIII) 1.0cm PROXIMAL TO BREAK SECTION
AREA INDICES DATA

OBS	COW_NO	LEG	TRT	CAI_2	EAI_2	XR1_2	PAI_2
1	168	L	1	731.01	625.02	938.44	497.41
2	168	R	1	900.51	624.66	925.75	533.78
3	169	L	1	901.24	602.07	981.03	562.77
4	169	R	1	1171.31	782.58	1024.00	633.95
5	170	L	1	762.78	688.10	1007.34	580.22
6	170	R	1	1169.71	784.22	1185.74	611.99
7	171	L	1	777.47	722.82	985.23	602.41
8	171	R	1	1018.16	724.66	992.56	587.36
9	172	L	1	990.66	667.43	1024.32	569.95
10	172	R	1	951.47	655.80	954.55	562.16
11	173	L	1	957.95	686.91	972.44	552.05
12	173	R	1	975.77	726.44	984.06	561.66
13	174	L	1	1001.28	602.00	975.10	506.69
14	174	R	1	951.47	616.39	975.24	514.79
15	276	L	2	1152.41	783.24	1111.21	620.23
16	276	R	2	1157.28	803.07	1145.24	628.89
17	277	L	2	1245.64	829.12	1273.78	666.00
18	277	R	2	1299.66	854.17	1295.68	668.62
19	278	L	2	1054.68	717.22	1058.20	626.53
20	278	R	2	1034.16	702.14	1056.36	618.47
21	279	L	2	1024.83	728.01	1008.40	627.94
22	279	R	2	1111.32	766.92	1102.08	597.53
23	280	L	2	1061.74	695.61	1095.40	578.07
24	280	R	2	1119.39	715.62	1094.40	561.03
25	281	L	2	1106.24	748.51	1166.78	579.51
26	281	R	2	1122.57	739.76	1106.42	607.12
27	282	L	2	1136.52	741.97	1203.81	607.87
28	282	R	2	1176.68	821.47	1112.78	624.79

THIRD METACARPAL (McIII) 1.0cm PROXIMAL TO BREAK SECTION
CHEMICAL ANALYSIS DATA

OBS	COW_NO	LEG	TRT	BMC_2	ASH_2	P_2	CA_2	MG_2
1	168	L	1	13.03	67.02	15.41	36.06	0.500
2	168	L	1	13.16	68.18	16.09	36.30	0.490
3	168	L	1	13.50	67.35	16.22	37.22	0.510
4	169	L	1	13.79	67.04	16.11	36.45	0.510
5	169	L	1	13.51	67.55	16.86	38.06	0.510
6	170	L	1	13.34	68.94	17.16	38.29	0.584
7	170	L	1	13.61	67.33	16.52	38.46	0.520
8	171	L	1	13.25	68.55	16.14	38.06	0.530
9	171	L	1	13.87	67.85	16.12	36.64	0.570
10	172	L	1	13.03	67.77	15.84	37.48	0.540
11	172	L	1	13.14	67.67	16.90	37.11	0.490
12	173	L	1	12.77	67.68	16.25	37.73	0.510
13	173	L	1	12.07	67.41	16.10	37.50	0.490
14	174	L	1	11.80	67.17	15.86	37.14	0.490
15	276	L	1	14.78	67.88	17.77	36.55	0.490
16	276	L	1	15.30	67.58	17.52	36.31	0.510
17	277	L	1	16.21	67.97	16.45	37.31	0.530
18	277	L	1	16.16	67.50	16.91	37.03	0.500
19	277	L	1	14.95	67.07	16.67	37.25	0.500
20	278	L	1	13.70	67.16	16.31	36.04	0.570
21	278	L	1	14.54	67.44	16.12	36.62	0.510
22	279	L	1	14.44	67.11	17.47	38.13	0.530
23	280	L	1	14.56	67.03	15.79	37.79	0.530
24	280	L	1	13.75	68.94	15.97	37.79	0.550
25	281	L	1	14.87	67.16	15.61	36.88	0.540
26	281	L	1	14.03	67.43	15.81	37.53	0.510
27	282	L	1	14.05	67.87	16.11	36.72	0.510
28	282	L	1	14.12	68.81	16.52	36.98	0.520

THIRD METACARPAL (McIII) 1.0cm PROXIMAL TO BREAK SECTION
NON-INVASIVE TECHNIQUES DATA

OBS	COW_NO	LEG	TRT	PIIMC_2	PHOTON_2	US_2	RBAE_2
1	168	L	1	13.5	1.617	3285.9	15.54
2	168	R	1	13.2	1.602	3378.1	15.26
3	167	L	1	14.6	1.644	3266.1	15.72
4	167	R	1	14.7	1.652	3169.8	15.86
5	170	L	1	14.1	1.616	3285.5	15.63
6	170	R	1	15.0	1.670	3351.2	15.23
7	171	L	1	14.2	1.723	3358.9	15.24
8	171	R	1	14.8	1.771	3358.9	15.22
9	172	L	1	13.3	1.626	3208.6	14.71
10	172	R	1	13.1	1.598	3195.8	15.31
11	173	L	1	14.6	1.727	3231.0	14.43
12	173	R	1	14.9	1.711	3264.4	14.73
13	174	L	1	12.6	1.530	3456.4	14.31
14	174	R	1	11.9	1.437	3421.2	14.11
15	276	L	2	16.2	1.822	3507.0	16.34
16	276	R	2	16.4	1.850	3448.2	17.17
17	277	L	2	16.4	2.014	3553.9	17.75
18	277	R	2	16.3	2.001	3485.5	17.77
19	278	L	2	14.8	1.781	3255.9	16.80
20	278	R	2	14.4	1.720	3264.3	15.98
21	277	L	2	15.4	1.824	3421.8	16.95
22	279	R	2	15.1	1.795	3374.5	16.63
23	280	L	2	15.0	1.754	3381.3	16.88
24	280	R	2	14.4	1.716	3418.4	16.54
25	281	L	2	14.0	1.688	3401.8	17.85
26	281	R	2	14.2	1.656	3440.1	16.48
27	282	L	2	14.8	1.749	3368.5	17.54
28	282	R	2	14.7	1.658	3358.1	16.37

Rib Cortical Bone Biopsy Data Codes

COW_NO = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
 2 = High phosphorus (.20% P, DMB)

PERIOD 1 = November 18, 1983
 2 = May 10, 1984

SPGR = Bone density ($\text{g}\cdot\text{cm}^{-3}$)
 ASH = Bone ash (%)
 MGP_CC = $\text{mg P}\cdot\text{cm}^{-3}$, fresh
 MGCA_CC = $\text{mg Ca}\cdot\text{cm}^{-3}$, fresh
 MGM_CC = $\text{mg Mg}\cdot\text{cm}^{-3}$, fresh
 P_ASH = Phosphorus as % of bone ash
 P_CM = Phosphorus as % of dry, fat-free bone
 CA_ASH = Calcium as % of bone ash
 CA_DM = Calcium as % of bone ash
 MG_ASH = Magnesium as % of bone ash
 MG_DM = Magnesium as % of dry, fat-free bone

RIB CORTICAL BONE BIOPSY DATA

OBS	COW_NO	TRT	PERIOD	SPGR	ASH	MGP_CC	MGCA_CC	MGM_CC
1	168	1	1	1.36598	56.7488	128.06	371.16	4.26
2	168	1	2	1.40660	57.2593	131.28	343.55	4.24
3	169	1	1	1.26746	55.1455	117.03	290.64	2.08
4	169	1	2	1.51090	62.5644	157.36	406.15	4.72
5	170	1	1	1.35086	52.0208	126.74	337.25	3.17
6	170	1	2	1.52524	61.1395	165.11	394.30	4.31
7	171	1	1	1.15670	47.4500	88.91	229.37	2.15
8	171	1	2	1.41725	61.7438	150.86	362.44	4.52
9	172	1	1	1.29909	60.6497	130.79	324.75	2.93
10	172	1	2					
11	173	1	1	1.27006	57.8307	121.85	307.23	2.66
12	173	1	2					
13	174	1	1	1.49278	60.1868	147.91	384.12	3.64
14	174	1	2	1.63171	63.8874	176.42	514.57	5.69
15	276	2	1	1.41051	62.0820	139.65	368.08	3.59
16	276	2	2	1.56694	63.4324	171.06	427.93	4.57
17	277	2	1	1.40687	61.9376	142.56	379.24	4.09
18	277	2	2	1.53771	64.7049	182.60	417.19	4.99
19	278	2	1	1.41676	62.1240	154.87	386.12	4.23
20	278	2	2					
21	279	2	1	1.55837	61.7566	165.70	416.69	4.36
22	279	2	2	1.49271	63.2688	153.37	414.09	6.04
23	280	2	1	1.53593	62.6221	164.47	399.61	4.55
24	280	2	2					
25	281	2	1	1.42706	61.7273	142.26	369.29	4.34
26	281	2	2	1.62010	64.6379	192.37	417.39	5.52
27	282	2	1	1.47762	61.1506	154.61	379.55	4.94
28	282	2	2	1.49201	61.8965	156.35	394.38	5.42

RIB CORTICAL BONE BIOPSY DATA									
UBS	COW_NO	TRT	PERIOD	P_ASH	P_DM	CA_ASH	CA_DM	MG_ASH	MG_DM
1	168	1	1	16.52	9.37	47.88	27.17	0.349	0.311
2	168	1	2	15.73	9.33	41.22	24.42	0.508	0.301
3	169	1	1	16.72	9.22	41.51	22.89	0.296	0.163
4	170	1	1	16.56	10.36	42.74	26.74	0.497	0.311
5	170	1	2	16.17	9.38	43.03	24.96	0.405	0.235
6	171	1	1	17.57	10.75	43.00	25.68	0.459	0.281
7	171	1	2	16.20	7.69	41.79	19.83	0.392	0.186
8	172	1	1	17.24	10.64	41.41	25.57	0.517	0.319
9	172	1	2	16.60	10.06	41.21	24.99	0.372	0.223
10	173	1	1	16.59	9.59	41.83	24.19	0.362	0.209
11	173	1	2	16.46	9.91	42.75	25.73	0.405	0.241
12	174	1	1	16.42	10.49	47.89	30.59	0.529	0.339
13	174	1	2	15.80	9.86	41.86	25.98	0.529	0.339
14	276	2	1	17.21	10.92	43.05	27.31	0.460	0.254
15	276	2	2	16.36	10.13	43.52	26.96	0.469	0.291
16	277	2	1	18.33	11.87	41.92	27.12	0.502	0.325
17	277	2	2	16.51	10.24	43.12	26.89	0.482	0.293
18	278	2	1	17.25	10.65	43.37	26.78	0.454	0.289
19	279	2	1	16.24	10.27	43.85	27.74	0.440	0.405
20	279	2	2	17.10	10.71	41.55	26.02	0.473	0.297
21	280	2	1	16.15	9.97	41.92	25.88	0.492	0.304
22	280	2	2	18.37	11.87	39.86	25.76	0.527	0.340
23	281	2	1	16.86	10.31	41.39	25.31	0.538	0.329
24	282	2	2	16.93	10.48	42.71	26.43	0.587	0.364

12th Rib Breaking Load Data Codes

COW_NO = Cow number

Side L = Left rib
R = Right rib

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

BL = Breaking load (kg)

12th RIB BREAKING LOAD DATA

OBS	COW_NO	SIDE	TRT	BL
1	168	L	1	184.0
2	168	R	1	173.00
3	169	L	1	170.00
4	169	R	1	132.55
5	170	L	1	99.55
6	170	R	1	65.00
7	171	L	1	88.55
8	171	R	1	77.55
9	172	L	1	112.55
10	172	R	1	106.00
11	173	L	1	109.55
12	173	R	1	127.00
13	174	L	1	76.00
14	174	R	1	80.00
15	276	L	N	134.00
16	276	R	N	135.00
17	277	L	N	155.00
18	277	R	N	120.55
19	278	L	N	149.00
20	278	R	N	162.00
21	279	L	N	186.00
22	279	R	N	187.55
23	280	L	N	184.55
24	280	R	N	173.00
25	281	L	N	159.00
26	281	R	N	157.00
27	282	L	N	125.00
28	282	R	N	125.0

Coccygeal Vertebrae Data Codes

COW_NO = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

V5 = Vertebra #5 (proximal)

V6 = Vertebra #6

V7 = Vertebra #7 (distal)

SG = Bone density ($\text{g}\cdot\text{cm}^{-3}$)

BMC = Bone mineral content (g)

ASH = Bone ash (%)

P = Phosphorus as % of bone ash

CA = Calcium as % of bone ash

MGP_CC = $\text{mg P}\cdot\text{cm}^{-3}$, fresh

MGC_CC = $\text{mg Ca}\cdot\text{cm}^{-3}$, fresh

MGM_CC = $\text{mg Mg}\cdot\text{cm}^{-3}$, fresh

COCCYGEAL VERTEBRAE DATA
VERTEBRA SIX (VI)

OBS	COM_NO	TRT	V6SO	V6BMC	V6ASH	V6P	V6CA	V6MG	V6MGP_CC	V6MOC_CC	V6MGM_CC
1	168	1	1.21	3.64	62.0	14.6	38.1	0.579	111.119	289.975	4.40671
2	169	1	1.19	3.51	63.0	13.7	38.2	0.553	102.709	286.385	4.14584
3	170	1	1.25	4.39	62.3	14.4	38.0	0.549	112.140	295.925	4.27534
4	171	1	1.18	3.20	64.2	14.8	38.3	0.576	119.694	291.661	4.36355
5	172	1	1.18	4.04	64.2	15.6	38.0	0.588	115.602	281.595	4.35732
6	173	1	1.21	3.15	64.2	15.2	38.2	0.571	118.077	296.745	4.43564
7	174	1	1.23	4.57	64.1	15.4	38.7	0.544	121.418	305.122	4.38905
8	276	2	1.34	3.27	64.0	14.9	36.9	0.502	127.782	314.739	4.30515
9	277	2	1.35	5.02	64.2	15.5	39.9	0.589	133.288	346.288	5.11281
10	278	2	1.38	3.92	64.4	15.1	35.7	0.638	134.879	325.812	5.65242
11	279	2	1.36	5.15	64.4	15.4	37.2	0.559	146.315	335.880	5.65935
12	280	2	1.47	3.84	64.4	15.9	36.5	0.615	146.818	362.345	5.10758
13	281	2	1.48	5.52	63.1	15.4	35.8	0.654	131.256	312.723	5.29427
14	282	2	1.17	3.52	64.	14.	35.	0.601			

COCYGEAL VERTEBRAE DATA VERTEBRA SEVEN (VII)											
OBS	COW_NO	TRT	V7SG	V7BMC	V7ASH	V7P	V7CA	V7MG	V7MGP_CC	V7MGC_CC	V7MGM_CC
1	168	1	1.23	3.09	63.1	14.2	38.1	0.552	110.210	295.706	4.28424
2	169	1	1.24	3.53	61.6	14.4	35.5	0.489	111.521	267.344	3.73318
3	170	1	1.25	3.78	64.1	14.6	35.9	0.588	115.145	300.502	3.54230
4	171	1	1.17	3.71	64.1	15.2	39.2	0.575	116.993	293.988	4.31983
5	172	1	1.24	3.51	62.3	14.6	37.4	0.603	116.189	304.373	4.45830
6	173	1	1.15	3.53	63.5	15.7	38.2	0.570	112.014	278.955	4.16242
7	174	1	1.20	3.63	63.5	14.5	37.5	0.535	113.544	291.084	4.07670
8	276	2	1.33	3.23	64.3	15.5	36.9	0.510	113.522	327.930	4.45985
9	277	2	1.36	3.48	64.4	15.1	36.9	0.532	113.656	325.591	4.47019
10	278	2	1.37	3.02	65.3	15.6	37.5	0.637	113.656	333.371	5.75529
11	279	2	1.42	3.17	62.7	15.7	37.5	0.557	117.443	329.162	4.87615
12	280	2	1.47	3.5	62.7	15.8	35.9	0.627	140.674	333.877	5.58243
13	281	2	1.35	3.4	64.9	15.8	35.7	0.556	137.592	328.386	5.10008
14	282	2	1.35	3.4	64.9	15.8	35.7	0.602	138.432	312.786	5.27442

Liver, Kidney, Heart and Muscle Data Codes

COW_NO = Cow number

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

L = Liver
K = Kidney
H = Heart
M = Muscle

P = Phosphorus, %, dry basis
Co = Cobalt, ppm, dry basis
Mn = Manganese, ppm, dry basis
Cu = Copper, ppm, dry basis
Zn = Zinc, ppm, dry basis
Fe = Iron, ppm, dry basis
Mo = Molybdenum, ppm, dry basis

OBS	COW_NO	TRT	KIDNEY DATA						
			K_P	K_CD	K_MN	K_CU	K_ZN	K_FE	K_MO
1	168	1	63.49.6	0.160	2.84	18.3	57.2	212.7	0.940
13	169	1	87.40.4	0.120	4.00	20.8	61.4	181.6	0.790
14	170	1	72.32.2	0.120	5.23	42.9	43.3	144.7	0.300
15	171	1	82.32.9	0.120	3.10	21.5	55.0	152.7	0.560
16	172	1	78.32.9	0.140	3.63	17.5	51.3	183.2	0.160
17	173	1	65.28.8	0.088	3.33	15.5	49.6	161.9	1.080
18	174	1	60.29.9	0.040	3.28	12.4	40.5	113.8	0.420
19	276	1	85.43.3	0.079	3.16	18.9	58.8	198.5	1.010
20	277	1	77.26.6	0.120	3.73	14.4	43.8	174.6	0.650
21	278	1	62.33.3	0.080	3.79	16.3	43.6	152.9	0.033
22	279	1	69.38.8	0.072	4.40	11.8	45.1	160.9	0.400
23	280	1	81.36.9	0.130	3.41	19.5	60.4	220.1	0.720
24	281	1	65.41.1	0.190	3.43	17.5	50.9	222.7	0.760
25	282	1	71.14.5	0.180	3.30	16.2	49.4	135.6	0.980

OBS	COW_NO	TRT	HEART DATA						
			H_P	H_CO	H_MN	H_CU	H_ZN	H_FE	H_MO
1	168	1	8268.4	0.070	0.996	19.0	71.20	167.6	0.110
1	169	1	8935.3	0.060	1.420	17.0	75.10	194.1	0.170
1	170	1	8303.4	0.041	1.270	16.0	62.90	196.4	0.170
1	171	1	5966.7	0.026	0.748	14.0	49.30	138.8	0.096
1	172	1	7742.8	0.041	1.410	17.0	59.60	191.2	0.240
1	173	1	7904.1	0.071	0.850	16.0	60.60	176.1	0.140
1	174	1	8758.0	0.061	1.370	16.0	61.30	196.4	0.110
1	276	1	6140.0	0.180	0.940	13.0	47.80	166.7	0.220
1	277	1	7608.8	0.083	0.840	17.0	58.80	165.7	0.140
1	278	1	8347.4	0.090	1.140	18.0	67.70	198.1	0.180
1	279	1	6740.3	0.058	1.070	15.0	56.80	147.9	0.061
1	280	1	7539.4	0.067	1.010	15.0	56.04	171.7	0.190
1	281	1	8061.1	0.096	1.520	16.0	59.90	182.7	0.100
1	282	1	6524.3	0.071	0.740	16.0	50.80	153.9	0.080

OBS	COW_NO	TRT	MUSCLE DATA						
			M_P	M_CD	M_MN	M_CU	M_ZN	M_FE	M_MO
1	168	1	2718.7	0.032	0.43	7.03	103.	95.7	0.110
2	169	1	4972.7	0.038	0.33	6.82	117.9	104.9	0.062
3	170	1	4383.9	0.019	0.41	8.75	120.	104.5	0.098
4	171	1	5473.4	0.028	0.47	8.60	126.3	118.3	0.070
5	172	1	6457.5	0.073	0.35	7.39	146.3	123.1	0.044
6	173	1	5755.2	0.016	0.33	7.29	144.2	123.9	0.081
7	174	1	5358.7	0.023	0.44	7.47	174.6	176.7	0.050
8	276	1	2999.4	0.498	0.33	6.09	117.	114.1	0.076
9	277	1	4182.7	0.044	0.35	6.00	138.	165.6	0.143
10	278	1	5662.8	0.058	0.30	6.88	138.	83.3	0.086
11	279	1	3793.6	0.024	0.49	6.48	146.	74.9	0.073
12	280	1	5148.4	0.044	0.40	6.94	148.	82.4	0.072
13	281	1	4584.4	0.053	0.49	6.04	120.	71.5	0.050
14	282	1	4252.9	0.044	0.34	6.94	111.1	103.4	0.029

Rumen and Abomasal Contents Data Codes

COW_NO = Cow number

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

R_ = Rumen contents

A_ = Abomasal contents

CA = Calcium, %, dry basis

P = Phosphorus, %, dry basis

MG = Magnesium, %, dry basis

K = Potassium, %, dry basis

ZN = Zinc, ppm, dry basis

MN = Manganese, ppm, dry basis

CU = Copper, ppm, dry basis

CO = Cobalt, ppm, dry basis

MO = Molybdenum, ppm, dry basis

OBS	COM_NO	TRT	ABOMASAL CONTENTS DATA									
			A_CA	A_P	A_MG	A_K	A_ZN	A_MN	A_CU	A_CO	A_MO	
1	168	1	0.405	0.314	0.060	0.358	92.3	15.2	7.55	0.392	5.74	
2	169	1	0.535	0.277	0.052	0.426	59.2	44.3	18.50	0.407	5.83	
3	170	1	0.921	0.420	0.071	0.519	48.1	63.3	39.10	0.951	10.74	
4	171	1	0.754	0.416	0.093	0.565	25.1	24.2	10.50	0.616	7.37	
5	172	1										
6	173	1										
7	174	1	0.543	0.309	0.043	0.347	70.4	72.4	18.00	1.070	5.73	
8	276	1	0.411	0.407	0.055	0.421	56.2	39.8	12.50	0.754	4.63	
9	277	2	0.733	0.462	0.107	0.414	25.1	51.7	15.20	1.150	4.26	
10	278	2										
11	279	2	1.620	0.853	0.264	0.985	48.6	94.2	20.30	1.440	13.88	
12	280	2										
13	281	2	0.981	0.450	0.141	0.512	43.9	46.8	13.40	1.090	9.77	
14	282	2	1.290	0.286	0.084	0.849	18.9	13.2	1.53	0.821	3.61	

Rumen Fluid Data Codes

COW_NO = Cow number

Trt 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

RF_P = Rumen fluid phosphorus ($\mu\text{g}\cdot\text{ml}^{-1}$)

RUMEN FLUID DATA

OBS	COW_NO	TRT	RF_P
1	168	1	356.3
2	169	1	339.3
3	170	1	61.6
4	171	1	353.6
5	172	1	311.6
6	173	1	413.4
7	174	1	72.3
8	276	2	128.1
9	277	2	275.4
10	278	2	448.2
11	279	2	220.5
12	280	2	525.9
13	281	2	104.5
14	282	2	221.4

Liver Biopsy Data Codes

COW_NO = Cow number

Trt 1 = Low phosphorus (.12% P, DMB)

2 = High phosphorus (.20% P, DMB)

P = Phosphorus, %, dry basis

CO = Cobalt, %, dry basis

MN = Manganese, %, dry basis

CU = Copper, %, dry basis

FE = Iron, %, dry basis

ZN = Zinc, %, dry basis

MO = Molybdenum, %, dry basis

OBS	COW_NO	TRT	P	CO	MN	CU	FE	ZN	MO
1	169	1	7241.8	0.327	8.45	141.9	258.6	62.8	2.41
2	170	1	8542.2	0.285	6.45	197.1	297.8	78.6	1.97
3	171	1	7481.1	0.420	6.02	166.3	266.5	87.1	1.87
4	172	1	7413.7	0.421	7.23	248.3	316.7	101.7	2.06
5	173	1	6585.3	0.280	4.58	191.0	129.0	50.0	0.95
6	174	1	7310.8	0.283	10.72	252.3	177.9	82.2	0.87
7	175	1	8734.4	0.484	6.15	192.3	176.1	74.2	0.87
8	276	2	7436.4	0.484	6.49	159.1	211.1	66.4	1.24
9	277	2	9102.1	0.600	7.09	327.6	359.3	88.3	1.24
10	278	2	8858.1	0.600	6.58	327.6	359.3	88.3	1.24
11	279	2	7168.4	0.342	5.41	143.1	212.1	67.1	0.30
12	280	2	8341.7	0.374	5.41	187.1	197.3	50.1	0.30
13	281	2	8713.9	0.391	3.69	207.8	162.3	78.3	2.43
14	282	2	8713.9	0.391	3.69	207.8	162.3	78.3	2.43

Feces Data Codes

ANIMAL = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

PERIOD 1 = November 18, 1983
2 = March 15, 1984
3 = May 10, 1984

FCA = Calcium, %, dry basis
FP = Phosphorus, %, dry basis
FMG = Magnesium, %, dry basis
FK = Potassium, %, dry basis
FZN = Zinc, %, dry basis
FMN = Manganese, %, dry basis
FCU = Copper, %, dry basis
FMO = Molybdenum, %, dry basis
FCO = Cobalt, %, dry basis

FECES DATA

OBS	ANIMAL	TRT	PERIOD	FCA	FP	FMO	FK	FZN	FHN	FCU	FMD	FCD
1	168	1	1	0.860	0.349	0.441	0.248	89.4	50.40	14.22	5.70	0.74
2	168	1	2	0.782	0.205	0.322	0.307	123.9	120.70	19.30	5.30	0.64
3	168	1	3	0.776	0.362	0.335	0.369	130.8	120.70	22.90	5.30	0.50
4	169	1	1	0.947	0.455	0.621	0.153	134.3	83.50	19.70	8.70	0.90
5	169	1	2	0.694	0.324	0.354	0.401	174.6	108.40	28.20	10.10	1.16
6	169	1	3	0.793	0.387	0.227	0.500	107.8	91.80	26.10	12.98	1.00
7	170	1	1	1.102	0.449	0.567	0.102	201.6	98.70	23.60	9.10	0.68
8	170	1	2	1.023	0.518	0.232	0.398	97.9	86.10	26.00	4.80	0.97
9	171	1	1	0.678	0.386	0.503	0.310	143.3	74.72	21.40	9.00	0.91
10	171	1	2	0.958	0.253	0.310	0.209	142.4	72.50	21.90	6.40	0.91
11	171	1	3	0.866	0.380	0.319	0.503	149.7	119.40	24.70	2.70	1.40
12	172	1	1	1.280	0.371	0.630	0.134	91.3	91.30	22.20	6.00	0.89
13	172	1	2	1.011	0.572	0.335	0.367	125.1	46.60	22.10	8.70	0.76
14	172	1	3	1.240	0.326	0.577	0.107	102.7	70.10	14.10	9.60	0.73
15	173	1	1	1.224	0.386	0.388	0.402	121.5	56.10	20.20	7.70	0.48
16	173	1	2	0.762	0.401	0.371	0.197	147.3	91.40	23.70	9.60	0.90
17	174	1	1	0.798	0.345	0.418	0.334	155.9	78.40	26.30	7.50	1.00
18	174	1	2	0.831	0.421	0.370	0.363	150.2	122.40	33.30	8.20	1.20
19	174	1	3	1.333	0.455	0.372	0.371	117.5	58.40	11.70	8.20	0.72
20	226	1	1	1.903	0.361	0.434	0.165	167.5	88.30	26.20	5.20	1.20
21	226	1	2	0.796	0.367	0.308	0.165	181.9	59.20	22.20	4.10	0.86
22	227	1	1	1.110	0.382	0.460	0.342	133.3	59.20	22.20	9.10	0.86
23	227	1	2	0.863	0.470	0.252	0.346	116.4	42.60	16.40	3.20	0.73
24	227	1	3	0.927	0.621	0.587	0.343	185.1	91.30	35.40	1.20	1.30
25	228	1	1	0.942	0.389	0.434	0.210	114.3	50.50	19.10	8.70	0.64
26	228	1	2	0.935	0.564	0.401	0.495	148.4	91.40	28.40	3.90	0.87
27	229	1	1	0.878	0.341	0.383	0.325	138.6	128.40	43.40	5.10	1.00
28	229	1	2	1.117	0.248	0.303	0.337	120.9	52.20	23.80	9.20	0.76
29	280	1	1	1.135	0.508	0.447	0.207	122.3	52.20	21.30	4.70	0.83
30	280	1	2	0.897	0.703	0.311	0.582	106.2	45.80	17.40	9.10	0.58
31	281	1	1	0.914	0.511	0.307	0.475	158.3	60.50	23.80	4.80	0.78
32	281	1	2	0.972	0.624	0.293	0.377	105.5	100.30	28.40	2.80	0.75
33	282	1	1	0.834	0.431	0.434	0.273	131.4	74.50	22.30	5.10	0.91
34	282	1	2	1.203	0.553	0.259	0.400	101.4	98.40	22.30	3.10	0.83

Hair Data Codes

ANIMAL = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

PERIOD 1 = November 18, 1983
2 = March 15, 1984
3 = May 10, 1984

HP = Phosphorus (ppm), dry basis
HZN = Zinc (ppm), dry basis

HAIR DATA					
OBS	ANIMAL	TRT	PERIOD	HP	HZN
1	168	1	1	161.5	116.4
2	168	1	2	154.9	98.4
3	168	1	3	157.7	72.1
4	169	1	1	114.7	98.7
5	169	1	2	185.4	112.9
6	169	1	3	207.7	139.2
7	170	1	1	138.1	106.5
8	170	1	2	156.5	106.6
9	170	1	3	157.9	115.7
10	171	1	1	170.7	119.1
11	171	1	2	155.5	100.6
12	171	1	3	167.0	113.7
13	172	1	1		
14	172	1	2	167.8	115.6
15	172	1	3		
16	173	1	1	170.5	128.4
17	173	1	2	92.7	81.7
18	173	1	3		
19	174	1	1	143.7	119.6
20	174	1	2	163.4	122.4
21	174	1	3	169.9	129.6
22	276	1	1	145.5	107.2
23	276	1	2	158.4	103.6
24	276	1	3	175.8	115.3
25	277	1	1	180.7	116.8
26	277	1	2	192.7	116.3
27	277	1	3	170.9	103.4
28	278	1	1	247.9	91.4
29	278	1	2	169.7	108.8
30	278	1	3		
31	279	1	1	124.7	107.3
32	279	1	2	132.1	113.7
33	279	1	3	116.3	88.8
34	280	1	1	149.9	106.6
35	280	1	2	124.3	111.6
36	280	1	3		
37	281	1	1	143.6	104.5
38	281	1	2	163.5	100.7
39	281	1	3	162.1	110.6
40	282	1	1	161.2	111.8
41	282	1	2	137.3	93.9
42	282	1	3	149.5	94.7

Saliva Data Codes

ANIMAL = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

PERIOD 1 = November 18, 1983
2 = March 15, 1984
3 = May 10, 1984

SALIVA_P = Phosphorus ($\text{mg P} \cdot \text{dl}^{-1}$)

SALIVA DATA				
OBS	ANIMAL	TRT	PERIOD	SALIVA_P
1	168	1	1	.
2	168	1	2	28.7
3	168	1	3	22.9
4	169	1	1	.
5	169	1	2	46.5
6	169	1	3	12.2
7	170	1	1	.
8	170	1	2	19.3
9	170	1	3	35.7
10	171	1	1	.
11	171	1	2	22.1
12	171	1	3	32.9
13	172	1	1	.
14	172	1	2	25.6
15	172	1	3	.
16	173	1	1	.
17	173	1	2	28.3
18	173	1	3	.
19	174	1	1	.
20	174	1	2	36.2
21	174	1	3	28.2
22	276	1	1	.
23	276	1	2	42.1
24	276	1	3	29.0
25	277	1	1	.
26	277	1	2	39.6
27	277	1	3	30.2
28	278	1	1	.
29	278	1	2	22.4
30	278	1	3	.
31	279	1	1	.
32	279	1	2	25.6
33	279	1	3	46.3
34	280	1	1	.
35	280	1	2	47.8
36	280	1	3	.
37	281	1	1	.
38	281	1	2	39.6
39	281	1	3	38.6
40	282	1	1	.
41	282	1	2	29.7
42	282	1	3	41.2

Day of Birth and Three-Week Postpartum Cow and Calf Data Codes

COW_NO = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

PER 1 = Day of birth
2 = 3-wk postpartum

COW_WT = Cow weight (lbs)
CALF_WT = Calf weight (lbs)
CALF_SEX = Gender
COW_HB = Cow hemoglobin ($\text{g}\cdot\text{dl}^{-1}$)
CALF_HB = Calf hemoglobin ($\text{g}\cdot\text{dl}^{-1}$)
COW_PCV = Cow packed cell volume (%)
CALF_PCV = Calf packed cell volume (%)

D = Dam
C = Calf

S = Serum
P = Plasma
WB = Whole blood

CA = Calcium ($\text{mg}\cdot\text{dl}^{-1}$)
P = Phosphorus ($\text{mg}\cdot\text{dl}^{-1}$)

OBS	COW_NO	TRT	PER	DPP	DPCA	CPP	CPCA	DWBP	DWBCA	CWBP	CWBCA
1	168	1	1	100	9	3	11	11	6	13	8
2	169	1	1	100	8	4	10	12	6	13	8
3	170	1	1	100	8	4	10	12	6	13	8
4	171	1	1	100	8	4	10	12	6	13	8
5	174	1	1	100	8	4	10	12	6	13	8
6	177	1	1	100	8	4	10	12	6	13	8
7	178	1	1	100	8	4	10	12	6	13	8
8	179	1	1	100	8	4	10	12	6	13	8
9	180	1	1	100	8	4	10	12	6	13	8
10	181	1	1	100	8	4	10	12	6	13	8
11	182	1	1	100	8	4	10	12	6	13	8
12	183	1	1	100	8	4	10	12	6	13	8
13	184	1	1	100	8	4	10	12	6	13	8
14	185	1	1	100	8	4	10	12	6	13	8
15	186	1	1	100	8	4	10	12	6	13	8
16	187	1	1	100	8	4	10	12	6	13	8
17	188	1	1	100	8	4	10	12	6	13	8
18	189	1	1	100	8	4	10	12	6	13	8
19	190	1	1	100	8	4	10	12	6	13	8
20	191	1	1	100	8	4	10	12	6	13	8

Day of Birth and Three-Week Postpartum Milk Data Codes

COW_NO = Cow number

TRT 1 = Low phosphorus (.12% P, DMB)
2 = High phosphorus (.20% P, DMB)

PERIOD 1 = Day of birth
2 = 3-wk postpartum

CA = Calcium, %, dry basis
MG = Magnesium, %, dry basis
K = Potassium, %, dry basis
NA = Sodium, %, dry basis
ZN = Zinc, ppm, dry basis
CU = Copper, ppm, dry basis
P = Phosphorus, %, dry basis

DAY OF BIRTH AND THREE WEEK POST-PARTUM MILK DATA										
OBS	COW_NO	TRT	PERIOD	CA	MG	K	NA	ZN	CU	P
1	168	1	1	0.848	0.155	0.172				
1	169	1	1	0.739	0.163	0.235	0.162	95	0.410	0.800
1	170	1	1	0.835	0.145	0.331	0.120	107	0.890	0.722
1	171	1	1	0.679	0.825	0.369	0.525	86	1.370	0.873
1	174	1	1	0.932	0.166	0.334	0.216	118	0.930	0.897
1	168	1	1	1.210	0.127	0.653	0.262	146	0.970	1.112
1	169	1	1	1.060	0.098	0.853	0.384	146	0.030	1.110
1	170	1	1	0.923	0.072	0.760	0.322	111	0.450	0.987
1	171	1	1	1.170	0.093	0.744	0.635	111	0.010	0.824
1	174	1	1	1.060	0.094	0.724	0.318	111	0.500	0.810
1	276	1	1	1.120	0.189	0.603	0.374	111	0.150	0.811
1	277	1	1	0.755	0.113	0.503	0.460	111	0.706	1.202
1	279	1	1	0.787	0.063	0.397	0.234	111	0.670	0.971
1	281	1	1	0.854	0.112	0.432	0.316	111	0.811	0.855
1	282	1	1	1.070	0.121	0.814	0.364	111	0.650	0.954
1	176	1	1	0.837	0.097	0.799	0.404	111	0.150	1.040
1	277	1	1	1.350	0.123	0.974	0.372	111	0.850	1.240
1	279	1	1	1.200	0.111	0.776	0.414	111	0.190	0.809
1	281	1	1	1.120	0.105	0.789	0.550	111	0.854	0.942
20	282	1	1	0.968	0.065	0.781	0.449	111	0.470	0.982
								111	0.740	1.110

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BIOGRAPHICAL SKETCH

Scot N. Williams was born on July 25, 1958, in Cleveland, Ohio. He attended Kenston High School in Bainbridge, Ohio, and graduated in June, 1976. He entered Broward Community College in August, 1976, and obtained an Associate of Art degree in May, 1978. He enrolled at the University of Florida in September, 1978, and completed the requirements for a Bachelor's degree in animal science in December, 1980.

Currently a candidate for a Ph.D. in animal science, the author has accepted a nutritionist position with Hoffmann-La Roche in Nutley, New Jersey.

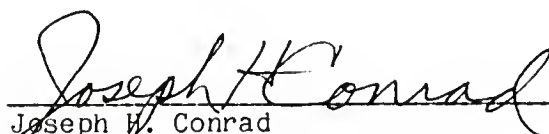
He was recently married to the former Vanessa Lee Berry and currently has no children--but plans to.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



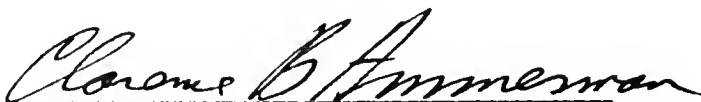
Lee R. McDowell, Chairman
Professor of Animal Science

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Joseph W. Conrad
Professor of Animal Science

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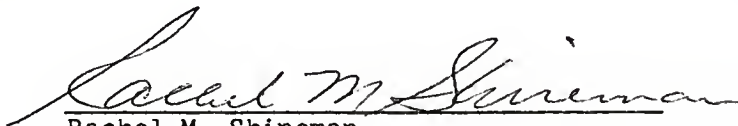


Clarence B. Ammerman
Professor of Animal Science

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Alvin C. Warnick
Professor of Animal Science

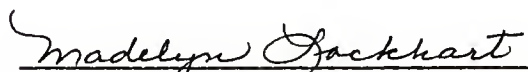
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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1987


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